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Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.01/A1

Topic: A.02. Stem Cells and Reprogramming

Support: the National Natural Science Foundation of China Grants 82325015
the National Key Research and Development Program of China 2021YFA1101800

Title: Construction of striato-nigral circuit via brain assembloids and human-mouse chimeric brain

Authors: *S. WU, Y. LIU;
Nanjing Med. Univ., Nanjing, China

Abstract: The abnormality of brain structure, function, or neural circuits leads to serious neurological and psychiatric diseases in learning, cognition, and movement. However, the limited access to human brain tissue, coupled with its unique structure and genetic background, makes it challenging to explore the neurological causes and pathogenesis of many brain disorders. Recently, based on human brain organoid technology, brain assembloids and further transplanted human-mouse chimeric brain provide human platforms for studying the interactions between cells-cells or regions-regions in the nervous system. To better understand its complexity across different brain regions, especially the formation of neural circuits between brain regions, we constructed striatal organoids and midbrain organoids, respectively. We then generated 3D striato-nigral assembloids by assembling striatal organoids with midbrain organoids. With AAV-hSYN-GFP-mediated viral tracing, extensive medium spiny neurons (MSNs) projections from the striatum to the midbrain are established, which formed synaptic connection with GABAergic neurons in midbrain organoids and showed the optically evoked inhibitory postsynaptic currents (oIPSCs) and electronic field potentials by labeling the striatum-like organoids with optogenetic virus. Furthermore, these striato-nigral assembloids exhibited enhanced calcium activity compared to that of individual striatal organoids. By transplanting the striatal organoids into the striatum of the severe combined immunodeficient (SCID) mouse to generate the human-mouse chimeric brain, we observed that the transplanted organoids could mature into functional MSNs after 3 months. Moreover, we found that the transplanted organoids were vascularized with host blood vessels and integrate into the host nervous system. Importantly, the transplanted organoids could not only send out projections to the substantia nigra of the midbrain in vivo, but also receive neural projections from host dopaminergic neurons, indicating the successful establishment of the human neural circuits in human-mouse chimeric brain by transplantation.Taken together, we successfully generated striato-nigral circuit model with human brain assembloids technologies and chimeric brain, which provides a platform to further explore more complex physiological and pathological phenotypes on neurological disorders

related to this circuit, including Huntington's disease, Parkinson's disease and addiction disorders.

Disclosures: S. Wu: None. **Y. Liu:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.02/A2

Topic: A.02. Stem Cells and Reprogramming

Support: Grant-in-Aid for JSPS Fellow

Title: Developing Strategies for Human Microglia-to-Neuron Conversion

Authors: *E. HATAI[1,3,4], K. MATSUDA[2], I. KATANO[5], R. TAKAHASHI[5], M. ITO[5], Y. SAKAI[3], K. NAKASHIMA[4], T. MATSUDA[2];
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Abstract: Introduction: Direct reprogramming of somatic cells into other lineages without going through an intermediate pluripotent state is a promising approach for manipulating cell fate, offering both speed and efficiency in generating specific cell types. We have previously shown that a single transcription factor, NeuroD1, can directly convert mouse microglia into neurons within a week. Furthermore, *in vivo* reprogramming of microglia in a stroke model mouse significantly improved neurological function. However, neuronal conversion of human microglia, which is critically important for clinical applications, has not yet been achieved. In this study, we attempt to address this gap.Methods & Results: Firstly, microglia were differentiated from human induced pluripotent stem cells (hiPSCs) and confirmed to have microglial characteristics based on gene expression patterns. While hiPSCs could differentiate into neurons with nearly 100% efficiency upon NeuroD1 expression, hiPSC-derived microglia (hiPSMG) were hardly converted into neurons despite high expression of NeuroD1. Therefore, we hypothesized that, unlike mouse microglia, a reprogramming barrier that inhibits the pioneer function of NeuroD1 is constructed during the differentiation of hiPSCs into human microglia. To explore the human-specific reprogramming barrier, we performed genome-wide CRISPR-Cas9 knockout screening in hiPSMG. As a result, random gene knockout allowed NeuroD1 expression to induce a small but detectable number of neurons from hiPSMG. We are currently investigating sgRNA enrichment in converted neurons to identify candidate barrier genes. We also plan to knock out these candidate genes in hiPSMG and assess neuronal conversion

efficiency via NeuroD1 expression. Additionally, we have established *in vivo*-like experimental platforms focused on human microglia, including brain organoids co-cultured with hiPSMG, and immunodeficient mice transplanted with hiPSMG. We will perform direct neuronal conversion in these *in vivo* conditions.
Conclusion: We have revealed that human microglia establish a reprogramming barrier against NeuroD1. We are actively working to identify and overcome this reprogramming barrier to enable successful neuronal conversion of human microglia. If successful, our study will provide critical insights into human microglial reprogramming and bring us one step closer to developing novel therapeutic strategies for neurological disorders.

Disclosures: E. Hatai: None. **K. Matsuda:** None. **I. Katano:** None. **R. Takahashi:** None. **M. Ito:** None. **Y. Sakai:** None. **K. Nakashima:** None. **T. Matsuda:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.03/A3

Topic: A.02. Stem Cells and Reprogramming

Support: JSPS Grant 23KJ1746

Title: Identifying Reprogramming Barriers in Somatic Differentiation via Rapid Neuronal Induction from Human iPSCs

Authors: *K. MATSUDA[1], E. HATAI[2,4,5], K. NAKASHIMA[6], T. MATSUDA[3];
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Abstract: Human induced pluripotent stem cells (iPSCs) exhibit exceptional plasticity, allowing for direct conversion into specific cell types upon forced expression of key transcription factors (TFs). However, as iPSCs begin differentiation toward somatic lineages, their reprogramming potential declines sharply, even when lineage-specific TFs are forcibly expressed. This suggests the existence of intrinsic barriers that emerge early in the differentiation process, but the molecular mechanisms underlying these barriers remain largely unknown. To address this, we established a robust system for rapid and highly efficient neuronal conversion from human iPSCs by introducing a defined combination of TFs. By generating single iPSC clones harboring these TFs, we achieved almost 100% conversion efficiency into functional dopaminergic-neurons, expressing tyrosine hydroxylase, upon doxycycline induction. Notably, the iPSC state can be

stably maintained in the absence of doxycycline, enabling precise temporal control of differentiation without the need for repeated TF delivery. Using this system, we conducted a genome-wide CRISPR activation (CRISPRa) screen to identify genes that act as reprogramming barriers during the transition from iPSCs to somatic cells. This screen uncovered key molecular roadblocks that limit reprogramming capacity in differentiating cells. Overall, this rapid and efficient induction system serves as a powerful platform for identifying reprogramming barriers in somatic differentiation. It also serves as a valuable tool for disease modeling and therapeutic research on disorders involving dopaminergic neurons reprogrammed from both iPSCs and somatic cells by forced expression of TFs, such as Parkinson's disease. Together, these findings offer new insights into the mechanisms restricting somatic cell reprogramming and establish a versatile approach for generating functional dopaminergic neurons from human iPSCs.

Disclosures: K. Matsuda: None. **E. Hatai:** None. **K. Nakashima:** None. **T. Matsuda:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.04/A4

Topic: A.02. Stem Cells and Reprogramming

Support: NIH R00HD096105
NSF RECODE 2225624
FRAXA fellowship
CIRM EDUC4-12822
CIRM EDUC2-12734
CIRM EDUC5-13637

Title: Anteroposterior organizers induced polarized neocortical organoids with areal signatures

Authors: *Y.-C. TSAI, M. WATANABE;
Univ. of California, Irvine, Irvine, CA

Abstract: Modeling neocortical arealization using human brain organoids is of growing interest. Here, we present a straightforward method involving short-term early exposure to rostral (FGF8) or caudal (BMP4/CHIR-99021) morphogens in human neocortical organoids. This treatment promoted the formation of anterior or posterior signaling centers, supporting long-lasting polarization. Single-cell RNA sequencing revealed distinct molecular signatures corresponding to cortical area-specific markers observed in human fetal cortex from published studies. To demonstrate the utility and physiological relevance of the polarized neocortical organoids, we modeled Fragile X Syndrome (FXS) using FXS neocortical organoids, which exhibited disrupted graded expression of SOX4 and SOX11 in the anterior-posterior axis, consistent with alterations reported in autism patient tissue. Together, our study provides a robust platform for generating

neocortical organoids with anterior-posterior polarization and highlights their relevance as a model of neurodevelopmental disorders with areal-specific phenotypes.

Disclosures: Y. Tsai: None. **M. Watanabe:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.05/A5

Topic: A.02. Stem Cells and Reprogramming

Support: 1 UM1 HG012651-01

Title: A reference panel of genetically diverse human iPSC lines to enable comparative cellular phenotyping

Authors: *E. PELLEGRINO, A. MELO CARRILLO, K. MERISOTIS, K. LEWKOWICZ, A. KENDRA, S. GRUBB, J. ALCOFORADO DINIZ, E. WERREN, J. GAROFALO, C. LORIG, P. ROBSON, W. SKARNES;
The Jackson Lab., Farmington, CT

Abstract: To build a comprehensive catalog of cellular phenotypes, it is essential to examine the influence of both genetic background and sex. We recently reprogrammed peripheral blood mononuclear cells (PBMCs) from 23 genetically diverse, healthy individuals and established 15 karyotypically normal clonal induced pluripotent stem cell (iPSC) lines—7 male and 8 female—for deep genomic and phenotypic characterization. We evaluated the differentiation capacity of these iPSC lines across multiple lineages, including mesoderm, endoderm, cortical brain organoids (CBOs), extra-embryonic mesenchyme (ExM), and primitive syncytium (PrS), followed by RNA sequencing (RNA-seq). Principal component analysis (PCA) revealed that most lines clustered closely, indicating a high degree of transcriptional similarity during differentiation. However, five lines exhibited lineage-specific differentiation defects. Notably, one of these lines harbored a splice site variant in exon 13 of the BCOR gene. BCOR mutations are common in iPSC lines and are associated with impaired differentiation potential. To evaluate the functional impact of this mutation, we are comparing the differentiation capacity and transcriptomic profiles of isogenic cell line pairs—one carrying the BCOR mutation and the other corrected using CRISPR prime editing. Another key focus of our work is the stability of X-chromosome inactivation (XCI) in female iPSC lines. We assessed H3K27me3 nuclear staining as a marker of inactive X chromosomes and tested various culture conditions to preserve XCI during long-term passaging. Preliminary results suggest that XCI is more stable in StemFlex™ medium compared to other commonly used media, including mTeSR™ Plus. Deep characterization of reference iPSC lines representing diverse genetic backgrounds of both sexes

provides a powerful platform to dissect how intrinsic variation influences lineage-specific differentiation and cellular phenotypes.

Disclosures: E. Pellegrino: None. **A. Melo Carrillo:** None. **K. Merisotis:** None. **K. Lewkowicz:** None. **A. Kendra:** None. **S. Grubb:** None. **J. Alcoforado Diniz:** None. **E. Werren:** None. **J. Garofalo:** None. **C. Lorig:** None. **P. Robson:** None. **W. Skarnes:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.06/A6

Topic: A.02. Stem Cells and Reprogramming

Title: A rapid, non-integrating, NGN2 mRNA-lipid nanoparticle platform for the generation of functional forebrain neurons from human pluripotent stem cells

Authors: M. KARIMI[1], S. DESHMUKH[1], G. BRAUN[1], C. ELLIS[1], ***M. E. SCHMIDT**[1], E. KNOCK[1,2], A. EAVES[1,3], S. A. LOUIS[1], A. HUNTER[1], R. JUDSON[1];
[1]STEMCELL Technologies, Inc., Vancouver, BC, Canada; [2]Simon Fraser Univ., Burnaby, BC, Canada; [3]BC Cancer, Terry Fox Lab., Vancouver, BC, Canada

Abstract: Overexpression of neurogenin-2 (NGN2) has been widely adopted to rapidly convert human pluripotent stem cells (hPSCs) into neurons. However, cell modification-based protocols are labor intensive, pose genomic integration risks, and can have viral safety requirements. To overcome these challenges, we developed the STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit, a next-generation system delivering rapid, efficient, and reproducible NGN2-driven neural differentiation. This integration-free approach uses mRNA-Lipid nanoparticle (LNP) delivery of NGN2 to generate neurons from hPSCs within six days. The kit was evaluated across four hPSC lines: H9, H1, WLS-1C and SCTi003-A. On day 6, the system achieved 96% ± 3% TUJ1-positive cells and yielded 7.6 × 10^5 ± 3 × 10^3 neurons per kit. After a further two weeks of maturation using BrainPhys™ hPSC Neuron Kit, 94% ± 2.6% of neurons expressed MAP2 and 90% ± 4.9% expressed the synaptic vesicle marker synapsin I (n = 8, 4 hPSC lines). Transcriptomic profiling confirmed a forebrain identity with high expression of FOXG1, TBR1, and SLC17A7 and single cell RNA sequencing showed the resulting neurons to be primarily glutamatergic with an increase in pre and post-synaptic proteins after 21 days in maturation medium. Electrophysiological analysis revealed spontaneous neuronal activity as early as day 8 (mean firing rate 0.11 ± 0.03 Hz), peaking at day 21 (1.5 ± 0.5 Hz), and persisting beyond 48 days, demonstrating sustained functionality. Addition of hPSC-derived astrocytes, generated using the STEMdiff™ Astrocyte Serum-Free Maturation Kit, further increased both the burst frequency and synchrony index compared to neurons alone (day 21 neurons alone burst frequency: 129.47± 35.77 and synchrony index: 0.09± 0.04; day 21 co-culture burst frequency: 190.53± 35.28 and synchrony index: 0.23± 0.02). STEMdiff™-TF Forebrain Induced Neuron

Differentiation Kit eliminates bottlenecks and offers a robust, easy-to-use, integration-free solution to rapidly and efficiently generate functional NGN2 neurons and accelerate neurobiological discoveries.

Disclosures: M. Karimi: A. Employment/Salary (full or part-time): STEMCELL Technologies, Inc. **S. Deshmukh:** A. Employment/Salary (full or part-time): STEMCELL Technologies, Inc. **G. Braun:** A. Employment/Salary (full or part-time): STEMCELL Technologies, Inc. **C. Ellis:** A. Employment/Salary (full or part-time): STEMCELL Technologies, Inc. **M.E. Schmidt:** A. Employment/Salary (full or part-time): STEMCELL Technologies Inc. **E. Knock:** 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. **A. Hunter:** A. Employment/Salary (full or part-time): STEMCELL Technologies, Inc. **R. Judson:** A. Employment/Salary (full or part-time): STEMCELL Technologies, Inc..

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.07/A7

Topic: A.02. Stem Cells and Reprogramming

Title: Establishing a functional standard for hiPSC-neural models used to screen for seizure liability with the multielectrode array (MEA) assay

Authors: *M. CLEMENTS[1], M. KREIR[2], N. MIYAMOTO[3], B. NODA[4], C. J. STROCK[5], I. SUZUKI[4,6], A. TUKKER[7], R. H. S. WESTERINK[8], T. YOSHINAGA[3];
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Abstract: Human induced pluripotent stem cell-derived (hiPSC) neurons are now established as a widely used model system in the safety pharmacology and disease modelling communities. The International Society for Stem Cell Research (ISSCR) recently released 'Standards for Human Stem Cell Use in Research', a document that outlines a set of recommendations that establish the minimum characterization and reporting criteria for working with human stem cells. The overarching goal of these criteria is to standardize practices, and in doing so, improve the quality of results. However, there are still no agreed-upon minimum acceptance criteria for the functional activity of hiPSC neuronal models. Rather, it is left to the end-user to determine

whether the electrophysiological phenotype of their cell model is fit for purpose. Over the last decade, the multielectrode array (MEA) field potential assay has become a popular tool for characterizing the electrical activity of hiPSC-neuronal batches, studying neural disease models, screening for new therapeutics, and evaluating drug-induced neurotoxicity. The hiPSC-neural MEA assay has been shown to be an effective high throughput method for screening seizure liabilities during the early stages of drug development. Drug-induced seizure liabilities have resulted in significant compound attrition during drug discovery. Consequently, the identification of seizurogenic effects earlier in this process is essential since it allows for selecting more favorable candidates before the start of costly preclinical *in vivo* studies. Despite the growing popularity of this seizurogenic MEA assay, there is still no recognized minimum acceptance criteria for the functional activity of the hiPSC-neural model used. But standardization will be required to assist data comparison and validation. The goal of this project is to set the minimum acceptance criteria for a spontaneous firing hiPSC-neural field potential seizurogenic assay for compound testing. Using our own experience with hiPSC-neurons in our respective labs in academia and in industry, our work with international consortia developing seizurogenic assays (HESI NeuTox and CSAHi), combined with published data, we have developed a proposed standard focused on hiPSC-neural spontaneous firing, network synchrony, and response to key positive controls.

Disclosures: M. Clements: A. Employment/Salary (full or part-time): Axion BioSystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Axion BioSystems. **M. Kreir:** A. Employment/Salary (full or part-time): Johnson & Johnson. **N. Miyamoto:** A. Employment/Salary (full or part-time): Eisai Co., Ltd. **B. Noda:** A. Employment/Salary (full or part-time): VitroVo Inc. **C.J. Strock:** A. Employment/Salary (full or part-time): Cyprotex US, LLC. **I. Suzuki:** A. Employment/Salary (full or part-time): VitroVo Inc.. **A. Tukker:** None. **R.H.S. Westerink:** None. **T. Yoshinaga:** A. Employment/Salary (full or part-time): Eisai Co., Ltd.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.08/A8

Topic: A.02. Stem Cells and Reprogramming

Support: NIH Grant R01-NS096012

Title: An independent evaluation of protocols for producing MGE progenitors from human pluripotent stem cells

Authors: *S. YOON, R. F. HUNT;
Univ. of California Irvine, Irvine, CA

Abstract: Transplantation of inhibitory neurons from the rodent embryonic medial ganglionic eminence (MGE) is a reliable preclinical strategy to regenerate interneurons in the damaged or diseased brain. Over the past 10 years, multiple protocols have emerged for manufacturing similar types of inhibitory neurons from human pluripotent stem cells, with the goal of advancing such a cell therapy product to the clinic for epilepsy and other neurological disorders. Here, we attempted to replicate and characterize three published derivation protocols for human MGE-like neurons that have been proposed for clinical translation. For comparison, we also simultaneously generated pan-neuronal progenitors and a modified protocol for producing human MGE-like interneurons. All five protocols were advanced in parallel at the same time, in the same lab and processed by the same lab personnel using a single cell line confirmed in house as high-quality and free of genetic abnormalities. Each protocol was carefully timed to produce an end product on the same day, and the resulting progenitors were processed for deep single-cell RNA sequencing (500M reads, PE 150, n = 4 biological replicates per protocol). Transcriptomic analysis revealed cell type and cell state expression profiles for each derivation protocol, and results were compared to the developing human brain. In separate experiments, human progenitors were collected, transplanted into hippocampus of immune compromised mice at P60 and the graft composition evaluated 6 to 9 months after transplantation. Our results show dramatic differences in reliability among derivation protocols, with no published protocol exclusively producing MGE progenitors. This suggests further rigorous refinement is needed before advancing an off-the-shelf human MGE cell therapy for epilepsy.

Disclosures: S. Yoon: None. **R.F. Hunt:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.09/A9

Topic: A.02. Stem Cells and Reprogramming

Title: A 3D Neurovascular Microfluidic Model of the Human Pluripotent Stem Cell-derived Blood-Brain Barrier

Authors: *K. XU[1], S. JANG[2], S. LIM[2], M. HENDRICKSON[1], K.-D. CHOI[1];
[1]BrainXell Inc., Madison, WI; [2]AIM Biotech, Singapore, Singapore

Abstract: The human blood-brain barrier (BBB) plays a critical role in maintaining central nervous system (CNS) homeostasis by precisely regulating the passage of molecules between the blood and brain parenchyma. Due to its physiological tightness, the BBB presents a significant obstacle to efficient drug transport into the CNS, which is a significant roadblock for the development of new treatments for CNS diseases. Hence, efficient and precise in vitro models of the human BBB are crucial for assessing drug design to enhance success rate of CNS-targeting candidates.Traditional BBB models often rely on primary cells, which pose limitations in

scalability, reproducibility, homogeneous cellular population and donor variability. To address these challenges, we developed a fully human induced pluripotent stem cell (hiPSC)-derived 3D BBB model incorporating iPSC-derived human brain microvascular endothelial cells (iHBMECs), -astrocytes (iACs), and -pericytes (iPCs) cultured within the idenTx 40 microphysiological system (MPS).We evaluated the structural and functional performance of this model by comparing it to a primary human cell-based 3D BBB cultured in the same platform. Barrier integrity was assessed using size-selective permeability assays with fluorescent tracers (70 kDa Dextran and 0.4 kDa FITC), demonstrating molecular weight-dependent transport. Immunolabeling for key cellular markers—including ZO-1, CD31, and Claudin-5 for endothelial cells; GFAP and S100β for astrocytes; and PDGFRβ for pericytes—confirmed appropriate cell identity and organization within the microvessel structures. Notably, the hiPSC-derived BBB model formed perfusable vessels and responded to pro-inflammatory cytokines with increased permeability, recapitulating key aspects of BBB dysfunction observed in disease. Furthermore, we demonstrated the potential of generating mature BBB through longer culture duration without compromising the characteristics and functionality of BBB phenotype.Our findings establish that a fully hiPSC-derived 3D BBB can closely mimic the structural and functional characteristics of primary human BBB models. This platform provides a scalable and physiologically relevant system for studying BBB dynamics and may serve as an effective tool for disease modeling and therapeutic screening in neurovascular research.

Disclosures: K. Xu: None. **S. Jang:** None. **S. Lim:** None. **M. Hendrickson:** None. **K. Choi:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.10/A10

Topic: A.02. Stem Cells and Reprogramming

Title: Building neural models from stem cells: a growth factor-driven approach

Authors: *J. KARANANKATTIL SUKUMARAN, S. SANKAR, T. SONTAG, D. SHANKAR;
Proteintech Group, Rosemont, IL

Abstract: Stem cells possess the unique ability to differentiate into any specialized cell type, making them an invaluable resource in both basic research and regenerative medicine. Differentiated stem cell models enable the investigation of proteins involved in developmental and disease processes—many of which are otherwise inaccessible due to the rarity or ethical inaccessibility of certain human tissues. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), when directed along specific lineages, offer robust systems for studying cell-type-specific proteins and functions. For example, three-dimensional midbrain organoids derived

from human pluripotent stem cells (hPSCs) have emerged as physiologically relevant models to explore human neurodevelopment and neurodegenerative diseases such as Parkinson's disease. In this study, we utilized defined combinations of human cell expressed growth factors demonstrating superior bioactivity and stability to drive hPSC differentiation toward regionally specified neural lineages. Differentiation efficiency and cell-type specification were monitored using a panel of well-validated antibodies targeting key developmental and neuronal markers. Our workflow highlights the critical role of precisely timed signaling cues and the use of high-quality reagents in achieving consistent and efficient neural differentiation. This strategy enables reliable characterization of stem cell-derived neural models and broadens their applicability across developmental studies, disease modeling, and therapeutic discovery.

Disclosures: J. Karanankattil Sukumaran: A. Employment/Salary (full or part-time): Proteintech. **S. Sankar:** A. Employment/Salary (full or part-time): Proteintech. **T. Sontag:** A. Employment/Salary (full or part-time): Proteintech. **D. Shankar:** A. Employment/Salary (full or part-time): Proteintech.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.11/A11

Topic: A.02. Stem Cells and Reprogramming

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

Authors: R. VAIDYANATHAN, C. SAVIC, M. DONEGAN, R. FIENE, ***J. LIU**, 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: R. Vaidyanathan: A. Employment/Salary (full or part-time): Fujifilm cellular dynamics. **C. Savic:** A. Employment/Salary (full or part-time): Fujifilm cellular dynamics. **M. Donegan:** 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. **C.B. Carlson:** A. Employment/Salary (full or part-time): Fujifilm cellular dynamics.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.12/A12

Topic: A.02. Stem Cells and Reprogramming

Title: Modeling Cetacean Neurodevelopment In Vitro to Explore Mammalian Cortical Evolution

Authors: *F. CANCELLOTTI, F. CREMISI;
Scuola Normale Superiore, Pisa, Italy

Abstract: Cetaceans possess some of the largest and most intricately folded brains among mammals, yet the developmental programs driving their cortical complexity remain largely unexplored. This project aims to establish in vitro models of cetacean neurodevelopment by applying induced pluripotent stem cell (iPSC) technologies to fibroblasts derived from two odontocete species with the highest degree of cortical gyrification: the orca (*Orcinus orca*) and the sperm whale (*Physeter macrocephalus*). The overarching goal is to investigate the emergence and role of basal radial glial cells (bRGs), key progenitors implicated in cortical expansion in mammals, and to assess their contribution to the evolution of neocortical architecture across

mammalian species. Advanced differentiation protocols will be employed to direct iPSCs toward neural lineages, with a particular focus on the development of supragranular neurons, which are critical for interhemispheric and cortico-cortical connectivity. Comparative analyses with human and mouse models will be conducted to identify conserved and lineage-specific mechanisms underlying cortical growth. Future integration of transcriptomic approaches is planned to further characterize cell populations and developmental trajectories. Cetaceans represent a striking example of convergent brain evolution, having independently developed complex cortical structures comparable to those of primates. Exploring their developmental programs in vitro provides a unique opportunity to identify general principles that govern brain expansion across mammals, as well as lineage-specific adaptations. Furthermore, the generation of cetacean iPSC lines constitutes a significant methodological advance, offering new tools for developmental and comparative neurobiology. In the long term, this research may inform not only evolutionary theory, but also strategies for species conservation and the study of brain vulnerability to environmental challenges.

Disclosures: F. Cancellotti: None. **F. Cremisi:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.13/A13

Topic: A.02. Stem Cells and Reprogramming

Title: Optimized Workflows for Modelling Human Pluripotent Stem Cell-Derived Neuron and Glial Interactions

Authors: J. CHAN[1], K. WRIGHTSON[1], A. MOOSA[1], M. KARIMI[1], ***J. OKUBO**[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 Agency, Vancouver, BC, Canada; [3]Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Co-cultures of human pluripotent stem cell (hPSC)-derived neurons with hPSC-derived microglia and astrocytes can recapitulate physiologically important neuron-glia interactions. However, generating sufficient yields of highly pure neurons, microglia, and astrocytes can be time consuming and technically challenging. To address this, we improved the cell yield and duration of our existing tri-culture workflow by using an improved microglia differentiation medium, integrating rapid NGN2-driven neuronal differentiation, and using cryopreserved astrocytes. Microglia were generated using STEMdiff™ Hematopoietic and Microglia Differentiation Kits and were characterized by flow cytometry for expression of CD45, CD11b, P2RY12, and TREM2. Prior to co-culture, microglial functionality was assessed by treating cells with 100 ng/mL lipopolysaccharide (LPS) for 24 hours. Their immune response was measured via qPCR for changes to homeostatic microglia marker expression and ELISA for

inflammatory cytokine secretion. hPSCs were generated and maintained using STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit and Forebrain Neuron Maturation Kit respectively. Cryopreserved SCTi003-A-derived astrocytes were maintained for one week and seeded onto the neurons at 5e4 cells/cm2 in STEMdiff™ Astrocyte Serum-Free Maturation Medium. The next day, microglia were seeded onto the co-culture at 2.5e4 cells/cm2 in BrainPhys™ hPSC Neuron Medium with STEMdiff™ Microglia Supplement 2. Tri-cultures were visualized by immunocytochemistry for GFAP+ astrocytes, MAP2+ neurons, and IBA1+ microglia. Microglia generated with the optimized differentiation medium displayed an average of 4.5 ± 2.0 fold expansion across four hPSC lines. They displayed an average of 85.7% ± 11.6% CD45+CD11b+ co-expression, 96.0% ± 4.3% P2RY12+, and 83.8% ± 13.9% TREM2+ expression by flow cytometry across 3 - 4 cell lines. Following LPS treatment, microglia showed a reduced expression of homeostatic microglial markers by qPCR ($p \leq 0.01$, $n = 3$) and secreted 5913 ± 1646 pg/mL CCL2 and 1066 ± 830 pg/mL IL-6 (mean ± SD) indicating that they are functional. After one week of tri-culture, all cell types exhibited expected morphologies. Stellate GFAP+ astrocytes co-localized with microglia's extended IBA1+ processes, which were wrapped around the healthy MAP2+ neuron dendrites. Our optimized protocol shortens the tri-culture workflow from 62 days to 45 days and yields functional microglia. These improvements enhance efficiency making the hPSC-derived tri-culture model more accessible for future studies.

Disclosures: J. Chan: A. Employment/Salary (full or part-time): STEMCELL Technologies. **K. Wrightson:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **A. Moosa:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **M. Karimi:** A. Employment/Salary (full or part-time): minoo.karimi@stemcell.com. **J. Okubo:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **A. Eaves:** A. Employment/Salary (full or part-time): STEMCELL Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 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

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.14/A14

Topic: A.02. Stem Cells and Reprogramming

Support: NIH Research Project Grant R01NS15276

Title: Lineage-traced ptbp1 depletion in mature astrocytes reveals distinct splicing alterations without neuronal features

Authors: *D. NIKOM[1], M. ZHANG[4], N. KUBOTA[2], A. ARIENT[4], S. SURINENI[3], S. ZHENG[3,5];
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Abstract: Astrocyte-to-neuron reprogramming via depletion of PTBP1, a potent repressor of neuronal splicing, has been proposed as a therapeutic strategy, but its efficacy remains debated. While some reported successful conversion, others disputed this, citing a lack of neuronal gene expression as evidence of failed reprogramming. This interpretation was further challenged, attributed to incomplete PTBP1 inactivation, fueling ongoing controversy. Mechanistic understanding of the conversion, or the lack thereof, requires investigating, in conjunction with lineage tracing, the effect of *Ptbp1* loss of function in mature astrocytes on RNA splicing, which has not yet been examined. Here, we genetically ablated PTBP1 in adult Aldh1l1-Cre/ERT2 Ai14 reporter mice to determine whether lineage traced *Ptbp1* knockout astrocytes exhibited RNA splicing alterations congruent with neuronal differentiation. We found no widespread induction of neurons, despite a minuscule fraction of knockout cells showing neuron-like transcriptomic signatures. Importantly, PTBP1 loss in mature astrocytes induced splicing alternations unlike neuronal splicing patterns. These findings suggest that targeting PTBP1 alone is ineffective to drive neuronal reprogramming and highlight the need for combining splicing and lineage analyses. Loss of astrocytic PTBP1 is insufficient to induce neuronal splicing, contrasting with its well-known role in other non-neuronal cells, and instead affects a distinct astrocytic splicing program.

Disclosures: D. Nikom: None. **M. Zhang:** None. **N. Kubota:** None. **A. Arient:** None. **S. Surineni:** None. **S. Zheng:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.15/A15

Topic: A.02. Stem Cells and Reprogramming

Title: Generating Enriched Authentic Midbrain Dopaminergic Neurons by Recapitulating Developmental Signaling Dynamics

Authors: *P. KANG, L. FENG, S.-C. ZHANG;
Ctr. for Neurologic Dis., Sanford Burnham Prebys, San Diego, CA

Abstract: Midbrain dopaminergic neurons (mDANs) originate from the floor plate (FP) which undergoes stepwise restriction to the medial FP and commitment to the dopaminergic fate. Protocols have been developed to derive midbrain FP cells from human pluripotent stem cells

that express FOXA2, OTX2, and LMX1A. However, these FP progenitors are often multipotent, resulting in heterogeneous differentiation to not only dopaminergic neurons but also various non-dopaminergic neurons, glial cells, and vascular leptomeningeal cell (VLMC)-like fibroblasts in vitro and particularly following transplantation, making it unpredictable for cell therapy. We sought to generate a progenitor population committed to the dopaminergic fate by recapitulating the embryonic signaling dynamics in vitro. We first induced a midbrain-specific FP identity by precisely modulating Wnt and Sonic hedgehog signaling to minimize contamination from forebrain and hindbrain cells. We then specified these cells toward the medial FP domain, the embryonic origin of mDANs, by identifying a novel molecular combination for human cells. Finally, we initiated neuronal program of the medial FP cells and successfully committed the progenitors to the dopaminergic fate by turning on the expression of MSX1. These dopaminergic progenitors (DAPs) rapidly gave rise to NURR1-, EN1-, and TH-positive mDANs within a few days upon removal of mitogens. scRNAseq revealed distinct molecular profiles for each stage of the progenitors, suggesting that stage-specific signaling cues govern lineage commitment. This approach offers a novel strategy for generating highly enriched authentic DAPs that give rise to predictable DANs in vitro and following transplantation, setting the foundation for safe and effective cell therapy for Parkinson's disease.

Disclosures: P. Kang: None. **L. Feng:** None. **S. Zhang:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.16/A16

Topic: A.02. Stem Cells and Reprogramming

Title: Functional analysis for the screening of iPSC-derived neural organoid response to neuroactive compounds

Authors: B. STREETER[1], D. SULLIVAN[1], *A. PASSARO[1], S. A. CHVATAL[1], D. C. MILLARD[2];
[1]Axion BioSystems, Atlanta, GA; [2]Axion Biosystems, Atlanta, GA

Abstract: *In vitro* neuronal models are valuable tools for studying brain function, neural diseases, and toxicology. Recent advances in induced pluripotent stem cell (iPSC) technology have allowed researchers to produce 3D models of neural tissue, termed neural organoids, that better recapitulate the cellular diversity and spatial architecture of *in vivo* tissue. Therefore, it is necessary to develop methods to accurately measure electrophysiology from these new, advanced 3D models. Here, traditional and novel microelectrode array (MEA) methods were used to characterize the electrical response of 3D neural models to neuroactive compounds. Pre-formed Human iPSC-Derived Midbrain Organoids (Catalog #200-0792 and Catalog #200-0793) from STEMCELL Technologies were cultured using the STEMdiff™ Neural Organoid

Maintenance Kit (Catalog #100-0120). The midbrain organoids were plated onto CytoView 6 MEA plates and, on Day 125 post-differentiation, dosed with neuroactive compounds or a DMSO control. Baseline and post-dose (1 hour after drug addition) recordings were taken using the Maestro Pro and showed robust changes in midbrain organoid activity patterns in response to the potassium channel blocker 4-aminopyrirdine and in response to the mitochondrial complex I inhibitor rotenone. Dosing with 4-aminopyrirdine increased the mean firing rate by 1.77 Hz and network burst frequency by 0.21 Hz, as compared to baseline, of midbrain organoids, while dosing with rotenone led to a decrease in both metrics (0.44 Hz decrease for mean firing rate and 0.24 Hz decrease for network burst frequency). Further, novel MEAs were created to facilitate more specialized measurement of 3D neural models on the Maestro Pro, including MEAs with high density electrodes (170 μm^2 area with a pitch of 50 µm) and MEAs with flexible, electrode-containing cantilevers. In total, this study shows that the Maestro Pro system can be used with both traditional and novel MEAs to study complex 3D neural models.

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

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.17/A17

Topic: A.02. Stem Cells and Reprogramming

Title: Comparative analysis of basement membrane extracts for human ipsc culture and induction into neural stem cells

Authors: *J. SILVA GARCIA[1], S. KATSOULIDIS[2], M. KENNEDY[3];
[1]Cell Biol., Thermo Fisher Scientific, Frederick, MD; [2]R&D, Thermo Fisher Scientific, Rockford, IL; [3]Thermo Fisher Scientific, Frederick, MD

Abstract: Advancements in human pluripotent stem cell (PSC) cultures have significantly improved with the adoption of coating matrices for feeder cell-free approaches. One commonly used matrix is derived from Engelbreth-Holm Swarm (EHS) mouse sarcoma, commercially known as Geltrex™ Flex or Matrigel®. These matrices support the self-renewal and pluripotency of PSCs and their induction into neural stem cells (NSCs). EHS matrices are complex protein extracts composed primarily of the basement membrane proteins laminin, collagen IV, and entactin, but also contain various growth factors. This heterogeneous composition is often cited as an underlying cause of variability. This study aims to compare the functionality and composition of different commercially available EHS matrices to understand

variations in cell culture performance. First, we expanded human PSC lines using different commercial matrices and assayed markers of pluripotency. Flow cytometry results following dual staining for stem cell markers (OCT4+/NANOG+) showed high expression (≥97%) across three EHS matrices. Further characterization by qRT-PCR showed a high expression level of genes associated with self-renewal and low expression of gene profiles associated with germ layer differentiation, confirming these cells remained undifferentiated across matrices. To assess if different matrices correlated with neural induction efficiency, PSCs were induced into NSCs. Flow cytometry following dual staining for Nestin and SOX1 showed high (≥96%) NSC marker expression independent of the matrix used. Immunocytochemistry against SOX1, SOX2, and PAX6,confirmed similar high induction efficiency (≥95%) across matrices. Lastly, we explored potential differences in matrix composition. Preliminary studies revealed a similar protein profile for the most abundant and high molecular weight proteins, laminin, collagen IV, and nidogen. Continuing efforts will compare low molecular weight and less abundant components, including growth factors, to help further characterize compositional differences. Overall, this study provides a functional and composition analysis comparison, indicating high comparability of various EHS matrices in PSC expansion and NSC induction workflows.

Disclosures: J. Silva garcia: A. Employment/Salary (full or part-time): Thermo Fisher Scientific. **S. Katsoulidis:** None. **M. Kennedy:** A. Employment/Salary (full or part-time): Thermo Fisher Scientific.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.18/A18

Topic: A.02. Stem Cells and Reprogramming

Title: The CellXpress.ai Automated Cell Culture System for automated, robust brain organoid generation

Authors: *S. GRUND-GRÖSCHKE[1], E. PICHLER[1], V. FISCHER[1], A. MICHLMAYR[1], E. KEIDEL[1], O. SIRENKO[2], F. SPIRA[1];
[1]Mol. Devices (Austria) GmbH, Puch bei Hallein, Austria; [2]Res., Mol. Devices LLC, San Jose, CA

Abstract: Culturing brain organoids presents several unique challenges starting with the maintenance of induced pluripotent stem cells (iPSCs), proceeding to seeding exact numbers of stem cells into target plates and adding differentiation and maturation media at exact time points to grow mature brain organoids. Maintaining stable culture conditions over several weeks and months is critical for generating mature organoids by having low variability between different wells and plates. Keeping brain organoids viable over extended periods requires daily feeding and fluid agitation to prevent the organoids from depleting the nutrients from their immediate

surroundings and to prevent organoid fusion. Using an in-incubator rocking system for media agitation, we were able to show that organoids on a rocker not only were similar in size, when compared to standard agitating methods, but also showed neuronal activity. Organoid health can be assessed by a built-in automated microscope for daily monitoring. The CellXpress.ai™ Automated Cell Culture System's cell journey feature enabled continuous tracking of each organoid from seeding through maturation, securing rigorous quality control and reproducibility. The CellXpress.ai system plays a key role in ensuring that the organoids are monitored, fed on time, and rocked during the phase in which they are rapidly consuming their surrounding nutrients. We established a streamlined protocol within the CellXpress.ai system. Here, stem cells were seeded into 96-well U-bottom plates for embryoid body formation and neural induction. For maturation, organoids were automatically transferred into 6-well plates and those plates were placed on rocking racks. The protocols included automated liquid handling with "smart media modules" for autonomous media exchange and imaging, automated drug screening, and quality control via automated integration of external devices such as the ImageXpress® Confocal HT.ai High-Content Imaging System. By using image-based health assessment of stem cell cultures, we were able to improve the culture quality by ensuring that the iPSC culture was in good condition. We were able to demonstrate that brain organoids generated on the CellXpress.ai system showed similar size and morphology to manual generated ones and exhibited neuronal activity This end-to-end solution demonstrates the feasibility of scalable, robust, and high-throughput brain organoid generation starting from healthy stem cells to mature brain organoids.

Disclosures: S. Grund-Gröschke: None. **E. Pichler:** None. **V. Fischer:** None. **A. Michlmayr:** None. **E. Keidel:** None. **O. Sirenko:** None. **F. Spira:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.19/A19

Topic: A.02. Stem Cells and Reprogramming

Title: Development of A Novel Small Molecule-Based Protocol for Directed Differentiation of hiPSCs to DRD2 Subtype Committed LGE Neural Progenitors

Authors: *M. BRYANT, A. KOKA, N. AMINI, K. SEARS, R. PADMANABHAN, J. JENSEN;
Trailhead Biosystems, Beachwood, OH

Abstract: Medium spiny neurons (MSNs) originate from the lateral ganglionic eminence (LGE) region of the developing forebrain and later migrate to the striatum where they mature into DRD1 and DRD2 neuronal subtypes. Loss of $DRD2^+$ cells due to HTT gene mutations is the cause of Huntington's disease (HD). HD is a devastating neurodegenerative disease with life

altering symptoms including motor, cognitive and psychological disorders. To this day there is no cure for HD. Effective *in vitro* differentiation of human stem cells to $DRD2^+$ MSNs would facilitate the development of Huntington's disease therapeutics by providing access to the relevant cell type with minimal contamination from other fates. Moreover, stem cell derived medium spiny neural progenitors have the potential to be transplanted into patients to replace lost MSNs. Here, we implemented High-Dimensional Design-of-Experiments (HD-DoE ®) to develop a small molecule-based differentiation protocol that can generate highly pure DRD2+ MSNs, closely following embryonic development. In stage 1, we developed a short protocol that guides hiPSCs to LGE-specific neural progenitor cells. These cells expressed forebrain markers such as *FOXG1*, *SIX3* and *OTX2*, ganglionic eminence markers *DLX5* and *ASCL1*, and LGE specific markers *FOXP1* and *FOXP2*. Culture purity was evaluated by immunocytochemistry, with an estimated 80%+ of cells committed to the LGE region. In stage 2, these LGE-specific NPCs were further differentiated to immature neurons, that had expression of DRD2 protein along with early neuronal markers such as TUBB3 and DCX. Flow cytometry analysis revealed that at least 90% of cells expressed FOXG1 and FOXP1, while 70% expressed SIX3 and CTIP2 markers. Importantly, expression of the medial ganglionic eminence (MGE) marker NKX2-1 protein was absent. To enable scale-up of DRD2+ neurons, this protocol was then validated in bioreactors for large-scale cell production, where we achieved 70% purity of DRD2-committed progenitors. These progenitors successfully differentiated into mature neurons that formed neural networks when plated. A key benefit of this novel protocol is its cost-effective translation to large-scale production, ensuring easy accessibility for researchers.

Disclosures: M. Bryant: A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **A. Koka:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **N. Amini:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **K. Sears:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **R. Padmanabhan:** A. Employment/Salary (full or part-time): Trailhead Biosystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Trailhead Biosystems. **J. Jensen:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.20/A20

Topic: A.02. Stem Cells and Reprogramming

Support: ICGEB Grant CRP/HRV23-05_EC
The Adris Foundation

Title: Uncovering the link between normal development and oncogenesis: the role of stem cells in understanding brain tumors

Authors: *K. KAPURALIN[1], K. MLADENIC[2], N. MOHOVIC[1], F. ALMAHARIQ[3];
[1]Fac. of Biotech. and Drug Develop., [2]Univ. of Rijeka, Rijeka, Croatia; [3]Clin. Hosp. Dubrava, Zagreb, Croatia

Abstract: Neural stem cells are group of progenitor cells, defined by their capacity for self-renewal, which can differentiate into particular neuronal or glial sub-types. The correct quantities and proportions of neural stem cells during central nervous system development and in adult life is precisely regulated. Failure of this rule may lead to tumor development. Glioma tumors derive from genetically aberrant cells with neuroglial stem cell-like self-renewing properties by a reactivation of different stem cell-like developmental gene programs, highlighting the numerous parallels that exist between developmental and oncologic mechanisms. Among gliomas there is inter-tumoral heterogeneity with respect of how aggressive tumor can be and intra-tumoral heterogeneity at the cellular level with different aberrant cell subtypes within the same tumor. It has been hypothesized that the source of cellular heterogeneity could be the dedifferentiation of tumor cells into a stem-cell like state. We are interested in understanding molecular mechanisms that regulate neural stem cells in normal brain and linking this mechanism with the brain cancer development. To investigate cell-specific changes in gliomas, we use multiomics single-cell sequencing technologies in combination with advanced bioinformatics tools and machine learning techniques. This approach is based on real clinical samples and includes functional validation in primary glioma cells derived from patients. All tumor tissues used in the experiments are obtained in accordance with ethical approvals. Sample collection are subject to strict ethical standards, with informed consent from patients. We are currently establishing a biobank of tumor tissues of different glioma subtypes in collaboration with a clinical center. We perform single-cell sequencing using the 10X Genomics platform to map cellular heterogeneity and to identify transcriptomic patterns associated with malignancy. Advanced machine learning algorithms are applied to identify and rank potential clinically relevant biomarkers. Functional validation is carried out in 3D primary glioma cultures derived from patients using CRISPR-Cas9 technology. Based on the integration of transcriptomic and phenotypic data, we will develop a predictive model to access the likelihood of tumor progression. This work aims to uncover novel factors and mechanisms that could potentially serve as new therapeutic targets capable of decreasing the malignancy of brain tumors.

Disclosures: K. Kapuralin: None. **K. Mladenic:** None. **N. Mohovic:** None. **F. Almahariq:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.21/A21

Topic: A.02. Stem Cells and Reprogramming

Title: Development of A Novel Directed Differentiation Protocol for Large-scale Production of hiPSC-Derived A9 Dopaminergic Neurons Subtype by Leveraging High Dimensional Design-of-Experiment (HD-DoE®) Technology

Authors: *A. KOKA[1], N. AMINI[1], M. BRYANT[1], K. SEARS[1], R. PADMANABHAN[2], J. JENSEN[1];
[1]Trailhead Biosystems, Beachwood, OH; [2]Trailhead Biosystems Inc., Beachwood, OH

Abstract: A9 dopaminergic neurons are a subtype of midbrain-derived dopaminergic neurons that reside in the substantia nigra pars compacta (SNc) of the brain. A9 neurons regulate motor function through dopamine release and have a characteristic slow and broad firing pattern in the brain. Research shows A9 dopaminergic neurons in particular are highly vulnerable in Parkinson's disease patients and by the time motor impairment appears in patients, around 40-50% of these cells are lost. Parkinson's Disease (PD) affected over 8.5 million people in 2019 and has the fastest growing population of affected individuals of any neurological disorder (WHO). While efforts have been made using mouse models to identify drugs and test cell therapies involving brain tissue grafts, one major barrier has been the lack of available human dopaminergic neurons for drug screening. We developed a novel protocol to generate human induced pluripotent stem cells (hiPSCs) derived A9 dopaminergic neurons using High-Dimensional Design-of-Experiments (HD-DoE®). The significance of this protocol is the successful generation of a highly pure SOX6+ population of dopaminergic neurons. SOX6 is a transcription factor that is only expressed in SNc dopaminergic neurons and not in ventral tegmental area (VTA) dopaminergic neurons, also known as A10 neurons. At the progenitor stage, the protocol yields more than 80% FOXA2/SOX6+ midbrain neural progenitor population. Upon terminal differentiation, a mature neuronal population is generated that expresses dopaminergic markers such as NURR1 (NR4A2), KCNJ6 (GIRK2) and TH along with mature pan neuronal markers such as SYN1 and MAP2. Moreover, the cells did not express CALB1, an A10 dopaminergic neuronal marker. The mature neurons have at least 70% purity as confirmed by detection of TH via flow cytometry, and they respond to KCl stimulation by releasing dopamine at higher levels than other commercially available cells. Other assays including immunocytochemistry, bulk RNA-seq, and RT-PCR were also used for thorough characterization of generated cells. This protocol was then adapted for large-scale manufacturing, and large numbers of cells were generated that maintained homogeneity and lineage as shown by specific marker expression. Accessibility to a functional pure population of A9 dopaminergic neurons with minimized batch to batch variation can significantly impact current efforts in drug screening, disease modeling and cell transplantation treatments for Parkinson's disease.

Disclosures: A. Koka: A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

excluding diversified mutual funds); ownership interest. **N. Amini:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **M. Bryant:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **K. Sears:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest. **R. Padmanabhan:** A. Employment/Salary (full or part-time): Trailhead Biosystems Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Trailhead Biosystems Inc. **J. Jensen:** A. Employment/Salary (full or part-time): Employment. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership interest.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.22/A22

Topic: A.02. Stem Cells and Reprogramming

Title: Automated Generation and Analysis of Midbrain Organoids using the CellXpress.ai Automated Cell Culture System

Authors: *E. PICHLER[1], S. GRUND-GRÖSCHKE[2], V. FISCHER[3], A. MICHLMAYR[3], E. KEIDEL[3], O. SIRENKO[4], F. SPIRA[3];
[1]Mol. Devices, Puch bei Hallein, Austria; [2]Mol. Devices (Austria) GmbH, Puch bei Hallein, Austria; [3]Mol. Devices (Austria) GmbH, Salzburg, Austria; [4]Res., Mol. Devices LLC, San Jose, CA

Abstract: Automated Generation and Analysis of Midbrain Organoids Using the CellXpress.ai Automated Cell Culture System Authors: Elisabeth Pichler, Sandra Grund-Groeschke, Verena Fischer, Astrid Michlmayr, Emilie Keidel, Oksana Sirenko, Felix Spira | Molecular Devices, LLC Human neurodegenerative diseases—such as Parkinson's disease which primarily affects the midbrain— pose a growing societal challenge. In recent years, the advent of brain organoids has revolutionized our ability to model these diseases in vitro. However, most midbrain organoid systems are labor-intensive and suffer from high variability between individual organoids, limiting their reproducibility and scalability. To address these limitations, we developed protocols for the generation of midbrain organoids using the CellXpress.ai™ Automated Cell Culture System. Starting from small molecule neural precursor cells (smNPCs), we implemented a fully automated differentiation protocol in a standard 96-well format that included seeding, feeding every other day, and daily imaging. The CellXpress.ai

system's cell journey feature enabled continuous tracking of each organoid from seeding through maturation, ensuring rigorous quality control and reproducibility. Midbrain organoids cultured on the CellXpress.ai system are homogeneous in morphology and size, leading to reduced inter- and intra-batch variability. As no matrix embedding is required, cumbersome manual handling can be circumvented, facilitating scalability. Midbrain differentiation was validated via whole-mount staining for key marker proteins. Neuronal activity was assessed indirectly through calcium imaging, which was visualized using the ImageXpress® Confocal HT.ai High-Content Imaging System. As proof of concept, we conducted an automated drug screening workflow, seamlessly integrating the CellXpress.ai system with robotic plate handling and high-content imaging. This end-to-end solution demonstrates the feasibility of scalable, reproducible, and high-throughput midbrain organoid generation and analysis. Our approach paves the way for more robust disease modeling and drug discovery in neurodegenerative research.

Disclosures: E. Pichler: None. **S. Grund-Gröschke:** None. **V. Fischer:** None. **A. Michlmayr:** None. **E. Keidel:** None. **O. Sirenko:** None. **F. Spira:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.23/A23

Topic: A.02. Stem Cells and Reprogramming

Title: Accelerated Maturation of hiPSC-Derived Oligodendrocytes Progenitor Cells Reveals Marker Transition and Myelination Capacity in 2D and 3D Models

Authors: C. FLIGOR[1], C. KANESKI[2], H. RUETH[2], A. JOHNSON[2], K. XU[2], *K.-D. CHOI[1]; [1]BrainXell, Madison, WI; [2]BrainXell Inc., Madison, WI

Abstract: Oligodendrocyte maturation and CNS myelination are essential for functional neural circuitry, and disruptions in these processes are central to many neurological diseases. Human iPSC-derived oligodendrocyte progenitor cells (OPCs) offer a robust platform to model human oligodendrogenesis in vitro. We developed an optimized differentiation protocol that yields highly pure OPC populations in under 30 days, significantly accelerating traditional timelines. These OPCs undergo progressive maturation, as demonstrated by the downregulation of progenitor markers PDGFRα and CSPG4, alongside the upregulation of mature oligodendrocyte markers such as MBP, PLP1, MOG, MAG, and Claudin-11, as confirmed by qPCR and immunocytochemistry. Additionally, highly purified populations of $O4^+$ OPCs (>95% purity by flow cytometry) began to express O1, indicating further progression toward maturation. In 2D co-culture with hiPSC-derived cortical glutamatergic neurons, OPCs differentiate into MBP+ oligodendrocytes that wrap TUJ1+ neurites, reflecting functional axon engagement. Notably, 3D brain organoids containing neurons, astrocytes, and OPCs provide an enriched microenvironment that further promotes oligodendrocyte maturation. Within this context, OPCs exhibit highly

arborized morphologies with extensive process outgrowth, more closely recapitulating in vivo oligodendrocyte architecture and suggesting enhanced integration into neural networks. Together, these results underscore the importance of multicellular context and dimensionality in driving OPC maturation. The combination of rapid OPC generation and co-culture, particularly in 3D organoids, offers a powerful platform for studying human myelination and supports high-throughput discovery of remyelination therapies.

Disclosures: C. Fligor: None. **C. Kaneski:** None. **H. Rueth:** None. **A. Johnson:** None. **K. Xu:** None. **K. Choi:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.24/A24

Topic: A.02. Stem Cells and Reprogramming

Support: Wake Forest Inst. Regen. Med. (NC state appropriation)

Title: A novel high-volume, low cost approach for 2-D microelectrode array assessment of iPSC-derived neurons

Authors: *B. M. ROEDER, A. WOEHR, A. ATALA, R. E. HAMPSON;
Wake Forest Inst. for Regenerative Med., Winston Salem, NC

Abstract: Immunohistochemical, genetic, and secretory biomarkers for neuron differentiation do not necessarily indicate the presence of electrophysiologically patent mature neurons. Verification of function also requires that the cells exhibit the electrophysiological characteristics of mature neurons. The use of a microelectrode array (MEA) plate with well allows for NPCs to be cultured in the well of the MEA for such verification of electrophysiological characteristics. In order to get sufficient number of replicants researchers are faced with the choice to balance the cost of multiple MEA plates to test cultures in parallel versus the time involved in reusing an MEA plate to test cultures in sequence. We aimed to determine if it was possible to test cultures in parallel while still using a single MEA plate by culturing NPCs outside of the MEA. Plastic microscope coverslips were coated in matrigel which allowed the NPCs to adhere to the coverslips. The NPCs were then incubated and allowed to mature. Coverslips were then tested at the desired time points of maturation using an Alpha Med Scientific MED64 Microelectrode Array (MEA) electrophysiology recording system attached to a Blackrock Neurotech recording system. To record the MEA was prepared with 200µL of DMEM, the coverslip was inverted and placed into the well of the MEA then gently pressed down so that the neurons were in contact with the electrodes, and the coverslip was covered with DMEM. Electrophysiological recordings were performed for 20 minutes. After allowing the cells to recover from the transfer and obtaining recordings of spontaneous activity, electrical and/or glutamate stimulation could be

applied in that order. After completion of recordings the coverslips were removed and discarded, and the MEA well washed to prevent cell adhesion to the MEA and in preparation for recording from another coverslip. These initial results were used to test maturation protocols and validate the maturation of NPCs into glutamatergic neurons. Spontaneous neural activity, electrically evoked, and glutamatergically evoked activity consistent with mature neurons were recorded using this method. The use of cultures on coverslips is a novel method that allows cost-effective electrophysiological testing of both novel culture methods prior to growing full cultures in an MEA and performing experiments that require injury or sacrifice of neural cultures in high volumes that would be either time or cost prohibitive using traditional methods. Additionally, this allows for the use of experimental conditions applied to the cultures that could potentially damage an MEA.

Disclosures: B.M. Roeder: None. **A. Woehr:** None. **A. Atala:** None. **R.E. Hampson:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.25/A25

Topic: A.04. Transplantation and Regeneration

Support: CIRM TRAN1-11548
NeoNeuron, LLC

Title: Neural Stem Cell Therapy for Traumatic Brain Injury in the Nonhuman Primate Model

Authors: B. J. CUMMINGS[1,3,4,5], E. W. DAADI[6], M. ARREDENDO[7], E. S. DAADI[6], T. OH[6], E. PARTIDA[2], ***M. DAADI**[7,8,9,10,11];
[1]Physical Med. & Rehab, Sue and Bill Gross Stem Cell Res. Center, Univ. of California Irvine, Irvine, CA; [2]Sue and Bill Gross Stem Cell Res. Center, Univ. of California Irvine, IRVINE, CA; [3]Anat. and Neurobiology, Univ. of California Irvine, Irvine, CA; [4]Inst. for Memory Impairments & Neurolog. Disorders, UCI, Irvine, CA; [5]Physical Med. & Rehabilitation, UCI, Irvine, Irvine, CA; [6]Southwest Natl. Primate Res. Center, Texas Biomed. Res. Inst., San Antonio, TX; [7]Univ. of Texas Hlth. San Antonio, Long Sch. of Med., San Antonio, TX; [8]Southwest Natl. Primate Res. Center, San Antonio, Texas Biomed. Res. Inst., San Antonio, TX; [9]The Sam & Ann Barshop Inst. for Longevity & Aging Studies, San Antonio, TX; [10]Glenn Biggs Inst. for Alzheimer's and Neurodegenerative Dis., San Antonio, TX; [11]Cell Systems & Anatomy; Radiology, Long Sch. of Med., San Antonio, TX

Abstract: Traumatic brain injury (TBI) is a significant cause of morbidity and mortality worldwide, with a greater risk of occurrence in athletes and military personnel. Cognitive and functional deficits following TBI are often life-altering and severely limit the quality of life in individuals living with a TBI. Direct mechanical damage causes alterations in neuronal and

vascular structures, leading to functional and structural changes in the blood vessels, gray matter, and white matter. Symptoms can then manifest as functional impairments like motor deficits, memory impairment, depression, sleep disturbances, and anxiety. A prospect in reversing functional deficits associated with TBI is neural stem cell (NSC) transplantation, which has been used as a method of promoting brain repair by replenishing the damaged cell pool and/or reducing neuroinflammation in several neurological disease models. We have previously reported that Shef-6 derived hNSCs engraft with host tissue in a rat model of brain injury to promote plasticity and functional recovery (Haus 2016, Badner 2021). We have now developed a novel marmoset animal model for the transplantation of NSCs to test neural repair of brain injury, utilizing a multi-faceted motor analysis and assessment of neurobehavioral improvement. Eighteen marmosets (6 vehicle, 4 with a dose of 1 million, and 8 with a dose of 5 million hNSCs) were utilized. This study demonstrated that Shef-6 hNSCs have promising potential to treat TBI in nonhuman primates by reducing sleep dysfunction, anxiety-like emotional disturbances, gait disability, and lesion volume, while also increasing white matter volume as assessed via MRI. The data also suggest that the effect of neural stem cell therapy is dose-dependent, as more robust functional recovery was observed in subjects receiving a 5 million NSC dose compared to those receiving the vehicle and 1 million hNSC dose. By optimizing the hNSC dose for treating TBI, a novel therapy could be applied to TBI patients to improve sensorimotor and non-motor dysfunctions.

Disclosures: B.J. Cummings: None. **E.W. Daadi:** None. **M. Arredendo:** None. **E.S. Daadi:** None. **T. Oh:** None. **E. Partida:** None. **M. Daadi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Marcel Daadi, NeoNeuron LLC.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.26/A26

Topic: A.02. Stem Cells and Reprogramming

Support: Wake Forest Inst. Regen. Med.

Title: Characterization of Human iPSC-Derived Forebrain Neurons for Organoid Modeling of Memory

Authors: A. L. WOEHR, B. M. ROEDER, A. ATALA, *R. E. HAMPSON;
Wake Forest Inst. Regen. Med., Winston-Salem, NC

Abstract: Organoid models are becoming increasingly useful models for both healthy and diseased tissues, as they recapitulate human genotypes and architectures. While scientists have developed models of other organs with success, no 2D culture or organoid model has attempted

to replicate the patterns of neural firing exhibited by the animal or human brains. Published research has demonstrated the feasibility of biocomputing systems using microelectrode arrays (MEAs) and cultured neurons . However, the electrical inputs for these models are not based on the firing patterns displayed in the human brain. Thus, we seek to create a model of human memory by adapting our existing blood brain barrier organoid model such that the contained neurons can be observed and manipulated electrophysiologically. To do so, the first necessary step is to reliably produce human iPSC-derived forebrain-type (glutamatergic and GABAergic) neurons.
The present work aimed to develop and refine a procedure for reliably producing and characterizing these neurons using immunohistochemistry, electrophysiology, and gene expression.
Using immunocytochemistry, we confirmed the expression of neuronal maturation markers MAP2, neurofilament heavy chain, beta-III tubulin, and PSD95. Gene expression of key glutamatergic and GABAergic neurons will be confirmed with qPCR. Additionally, using a microelectrode array, we demonstrated that these cells produce waveforms comparable to those recorded from in vivo human electrophysiology studies.
We conclude that these cells can be incorporated into a multi-cell type organoid to recapitulate patterns of human memory.

Disclosures: A.L. Woehr: None. **B.M. Roeder:** None. **A. Atala:** None. **R.E. Hampson:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.27/A27

Topic: A.02. Stem Cells and Reprogramming

Support: NSF Grant 445133-31003 FG22937

Title: Investigating how genetic and pharmacologic manipulations of N-glycan branching interact to impact neural stem cell fate bias

Authors: *L. NISHITANI[1], L. TENNIS[1], L. A. FLANAGAN[2];
[2]Neurol., [1]Univ. of California, Irvine, Irvine, CA

Abstract: Neural stem and progenitor cells (NSPCs) are multipotent and differentiate into the brain's main cell types: neurons, astrocytes, and oligodendrocytes. NSPCs hold great potential to treat neurological conditions, but current therapies are limited by the inability to predict NSPC fate prior to transplantation. Understanding mechanisms governing NSPC differentiation is therefore important for improving NSPC treatments. N-glycosylation is the process where carbohydrates, called glycans, are added to proteins destined for the cell surface. Previous research identified the glycosyltransferase MGAT5 as a key regulator of NSPC fate. MGAT5

activity produces branched N-glycans, and treating cells with N-acetylglucosamine (GlcNAc) metabolically promotes N-glycan branching. However, much remains unknown regarding the interplay of MGAT5 and GlcNAc treatment in NSPC fate and whether GlcNAc affects other forms of glycosylation. This study therefore examines how GlcNAc alters NSPC fate by assessing its effects on NSPC differentiation and N-glycan expression in Mgat5 wildtype (WT) and knockout (KO) mouse NSPCs (mNSPCs). Immunocytochemistry revealed that GlcNAc treatment increases astrogenesis in Mgat5-KO mNSPCs, but does not appear to affect neurogenesis. Furthermore, preventing GlcNAc from incorporating into the N-glycan pathway does not block its effects on astrogenesis in mNSPCs lacking Mgat5. Flow cytometry showed that GlcNAc altered other forms of N-glycosylation, such as fucosylation and sialylation, in both Mgat5-WT and -KO mNSPCs. These findings suggest that GlcNAc modulates other forms of glycosylation and may have effects on NSPC fate independent of MGAT5 and the N-glycan branching pathway, offering novel insights into the roles of glycans in NSPC differentiation and neural development.

Disclosures: L. Nishitani: None. **L. Tennis:** None. **L.A. Flanagan:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.28/A28

Topic: A.02. Stem Cells and Reprogramming

Support: AFOSR grant FA9550-22-1-0405

Title: Actin dynamics and impact of actin arrest on calcium dynamics in developing hNPCs

Authors: *S. J. GATES, III[1], K. M. O'NEILL[2], W. LOSERT[3];
[1]Inst. for Physical Sci. and Technol., Univ. of Maryland Col. Park, College Park, MD; [2]A. James Clark Sch. of Engin., Univ. of Maryland, Silver Spring, MD; [3]Col. of Computer Mathematical and Natural Sci., Univ. of Maryland, College Park, MD

Abstract: Beyond traditional electrical and chemical signaling, oscillations play a key role in brain information flow and processing. Recent work highlights the importance of mechanical signaling within the cytoskeleton, which can exhibit self-sustaining waves and oscillations. In this study, we investigated the dynamics of actin polymerization using LifeactGFP in a human neural precursor cell (hNPC) model during key differentiation stages: undifferentiated, naïve (4-10 days post-differentiation), and mature (14-28 days post-differentiation). The hNPC model chosen creates mixed populations, containing mostly astrocytes and some neurons. We analyzed actin throughout development using optical flow to quantify dynamics. We found that the size and localization of actin dynamics appears to adjust to match well-established functional needs throughout differentiation. These changes may enable the initiation and elongation of processes,

and ultimately the formation of synaptic and perisynaptic structures. Critically, in all developmental stages, actin remains dynamic within the cell's body. Moreover, using naïve differentiated cells, we explored how actin arrest may impact calcium signaling within our hNPC system. Using fluorescent imaging with a calcium indicator, Calbryte, we visualized calcium at 3 Hz, which allowed us to capture most of the slower timescale dynamics in the populations. We found that actin arrest shortens the duration while increasing the frequency of detected calcium events, leading to decreases in the time-delay cross-correlation in the networks. This work suggests that actin may act to dampen calcium signals, where arrest may disrupt this dampening, leading to increased excitability. Therefore, mechanochemical interventions can impact calcium signaling and are thus an interesting regime to continue future research focused on neural signaling and communication.

Disclosures: S.J. Gates: None. **K.M. O'Neill:** None. **W. Losert:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.29/A29

Topic: A.02. Stem Cells and Reprogramming

Support: ANID-FONDECYT 1230454 (LV-N)
ANID-FONDECYT 1250955 (FJB)
ANID-MILENIO (NCN2023_032, to LVN and FJB)
ANID 21253135 (SG-J)

Title: R-spondin 2 signaling regulates proliferation of NSCs in the adult mouse hippocampus

Authors: *S. GUTIERREZ-JIMENEZ[1], D. VALENZUELA-BEZANILLA[2], S. B. ARREDONDO[2], S. H. SANTIBAÑEZ[2], F. J. BUSTOS[2], L. VARELA-NALLAR[2];
[1]Inst. of Biomed. Sciences, Univ. Andrés bello, Santiago, Chile; [2]Inst. of Biomed. Sciences, Univ. Andrés bello, Santiago, Chile, Univ. Andrés Bello, Santiago, Chile

Abstract: Adult hippocampal neurogenesis in the dentate gyrus (DG) is tightly controlled by the neurogenic niche. The Wnt/β-catenin pathway is a key regulator of neural stem/progenitor cell (NSPC) proliferation and fate-commitment. In stem cells from adult peripheral tissues, R-spondin (RSPO) proteins, ligands of LGR receptors, potentiate Wnt signaling activity to regulate stem cell proliferation. Here, we investigated whether RSPO/LGR signaling regulates the proliferation of neural stem cells in the adult mouse hippocampus. We found that RSPO1-3 are expressed in the DG and in primary cultures of DG astrocytes, which are a crucial component of the neurogenic niche. Cultured hippocampal NSPCs expressed LGR4-6 receptors, and their expression decreased upon differentiation, concomitantly with a reduction in Cyclin-D1 and an increase in proneural gene expression. Co-culture of NSPCs with DG astrocytes induced NSPCs

proliferation, which was prevented by knocking down RSPO2 expression in astrocytes or LGR5 expression in NSPCs. Moreover, RSPO2 knockdown in the DG of 2-month-old mice by stereotaxic injection of lentivirus expressing shRNA targeting RSPO2 reduced the density of proliferating cells expressing the mitotic marker Ki67. Immunostaining with specific markers in combination with Ki67 revealed that RSPO2 knockdown specifically reduced proliferation of NSPCs, without affecting the proliferation of neuroblasts. Our findings demonstrate that the RSPO/LGR module is expressed in the adult mouse DG, and that RSPO2 is a component of the signaling mechanisms within the dentate niche that regulate neural precursor cell proliferation.

Disclosures: S. Gutierrez-Jimenez: None. **D. Valenzuela-Bezanilla:** None. **S.B. Arredondo:** None. **S.H. Santibañez:** None. **F.J. Bustos:** None. **L. Varela-Nallar:** None.

Poster

PSTR369: Pluripotent Stem Cell Neuronal Differentiation, Specification, Organoids, and Assembloids

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.30/A30

Topic: A.07. Development of Neural Systems

Support: NSF Grant CNS-1730158
NSF Grant ACI-1540112
NSF Grant ACI-1541349
NSF Grant OAC-1826967
NSF Grant OAC-2112167
NSF Grant CNS-2100237
NSF Grant CNS-2120019

Title: Protosequences in brain organoids model intrinsic brain states

Authors: *T. VAN DER MOLEN[1,2], K. S. KOSIK[2], T. SHARF[1];
[1]Univ. of California, Santa Cruz, CA; [2]Univ. of California Santa Barbara, Santa Barbara, CA

Abstract: Neuronal firing sequences are thought to be the basic building blocks of neural coding and information broadcasting within the brain. However, when sequences emerge during neurodevelopment remains unknown. We demonstrate that structured firing sequences are present in spontaneous activity of human and murine brain organoids and ex vivo neonatal brain slices from the murine somatosensory cortex. We observed a balance between temporally rigid and flexible firing patterns that are emergent phenomena in human and murine brain organoids and early postnatal murine somatosensory cortex, but not in primary dissociated cortical cultures. Our findings suggest that temporal sequences do not arise in an experience-dependent manner, but are rather constrained by an innate preconfigured architecture established during neurogenesis. These findings highlight the potential for brain organoids to further explore how exogenous inputs can be used to refine neuronal circuits and enable new studies into the genetic

mechanisms that govern assembly of functional circuitry during early human brain development. (in press, Nature Neuroscience)

Disclosures: T. van der Molen: None. **K.S. Kosik:** None. **T. Sharf:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.01/A31

Topic: A.06. Developmental Disorders

Support: Simons Foundation Autism Research Initiative 899599
UCSF Physician Scientist Scholars Program
Humboldt Foundation
Max Planck Society

Title: Differential utilization of hippocampal learning strategies as a source of individual differences within wild-type rats and those with a mutation to an ASD risk gene

Authors: C. Y. LEE[1], N. YOKOTA[1], C. HOLOBETZ[1], G. WILLIAMS[1], M. MEHRA[1], A. CHAUDHRY[1], J. TON[1], P. DAYAN[2], *D. B. KASTNER[1];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Max Planck institute for Biol. Cybernetcs, Tuebingen, Germany

Abstract: Learning is a dynamic process that varies widely across individuals, often involving distinct strategies. To understand this variability, we conducted two analyses of the behavior of rats performing a task requiring spatial alternation among specific triplets of arms in an environment containing six arms in a row.
In the first analysis, we compared rats with bilateral hippocampal lesions (n = 50) to rats that underwent control surgery (n = 55). Using the data-driven choice-wide behavioral association study (CBAS) method, we identified learning strategies that were distinguished by sequences of arm visits that differed between the two groups. Control rats more frequently moved in consistent directions across the arms of the track, progressively narrowing down their choices to visit just the correct arms. In contrast, at least early in learning, hippocampal-lesioned rats were less likely to continue in a consistent direction.
This group-level contrast defines partial strategies of control- and lesion-associated sequences of arm visits. However, individual learning trajectories were very variable. To test the hypothesis that individual wild-type (WT) animals might rely to different degrees on their hippocampus to perform the task, we conducted a second analysis on a larger cohort of WT rats (n > 200). Consistent with the hypothesis, individuals varied in their use of control- and lesion-associated sequences, with some WT rats exclusively favoring lesion-associated choices, despite having an intact hippocampus.
If differential hippocampal utilization is a source of inter-animal variability, it could also be

modulated by psychiatric risk genes as a source of pathogenicity. We found that rats haploinsufficient for *Scn2a* (n > 200), a high-confidence autism spectrum disorder (ASD) risk gene, are more biased toward lesion-associated strategies than WT rats, and therefore potentially less reliant on the hippocampus.
Together, these findings suggest that the hippocampus supports one or more specific learning strategies, but that individual animals can vary in their reliance on these strategies. Characterizing learning at the individual level may yield a better understanding of the neural basis of flexible and impaired learning.

Disclosures: C.Y. Lee: None. **N. Yokota:** None. **C. Holobetz:** None. **G. Williams:** None. **M. Mehra:** None. **A. Chaudhry:** None. **J. Ton:** None. **P. Dayan:** None. **D.B. Kastner:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.02/A32

Topic: A.06. Developmental Disorders

Support: Thuthuka Grant (National Research Foundation, South Africa, No: BM_TTK190827471815)
Female Academic Leaders Fellowship (FALF)
Hellen Iris Ellen Hodges Stress and Emotional Problems Grant

Title: Autism-like behaviors in Sprague-Dawley pups following gestational cadmium-induced hypertension

Authors: *B. C. MASEKO, M. GOUWS;
Anatom. Sci., Univ. of the Witwatersrand, Johannesburg, South Africa

Abstract: Cadmium chloride pollution is increasing with globalisation, and is known to cause preeclampsia complications in pregnancy. Preeclampsia is a dangerous condition characterised by hypertension and proteinuria, and is linked to cadmium chloride exposure. The condition is increasingly being linked to autism in progeny. Autism poses significant challenges and is marked, among other factors, by restricted, repetitive behaviours and impaired social skills. It is still not understood what leads to autism; however, the neurodevelopmental process is a large culprit, with preeclampsia increasingly preceding many cases. This study aimed to induce preeclampsia in pregnant Sprague Dawley rats via cadmium chloride exposure and thereafter assess the ultimate effects on developing offspring with respect to behavioural neurodevelopmental outcomes. Assessment of pregnant dams revealed evidence of preeclampsia in the exposed dams, which was further supported by the observation of restricted intrauterine growth in exposed litters upon birth. When conducting pup behavioural analysis, we observed, among other signs, reduced exploration, heightened anxiety and repetitive behaviours, all hallmark features of autism. We thus conclude that *in utero* exposure to cadmium induces

autism-like ideation through preeclampsia in Sprague-Dawley rat offspring. Our comprehensive series of behavioural essays confirms autism-like behaviours in offspring following exposure to cadmium, motivating closer monitoring of enviornmnetal cadmium levels and exposure minimisation to prevent the trigger for autism in offspring.

Disclosures: B.C. Maseko: None. **M. Gouws:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.03/A33

Topic: A.06. Developmental Disorders

Title: Effects of a novel social conditioning paradigm in BTBR T^+ $Itpr3^{tf}$/J mice

Authors: *B. BECHER[1], F. M. PADILLA[2], N. AHMED[3], O. L. SMITH[4], T. SCHACHTMAN[3], D. Q. BEVERSDORF[5];
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Abstract: Autism Spectrum Disorder (ASD) is characterized by difficulties with social interaction, as well as repetitive and restricted patterns of behavior. Applied Behavior Analysis (ABA) therapy, which uses principles of reinforcement to shape behavior, remains one of the most empirically supported approaches for improving functional independence in individuals with ASD. However, despite its widespread use, responses to ABA-based interventions vary widely, suggesting that individual factors—such as ASD etiology—may influence treatment effectiveness. This study aimed to model an ABA-style intervention in a preclinical setting to allow for further exploration of which individuals might benefit most. To do this, we developed a novel social conditioning paradigm designed to model one of the most common techniques in ABA therapy: pairing a desirable behavior with a rewarding stimulus. This paradigm was tested in BTBR T^+ $Itpr3^{tf}$/J (BTBR) mice, a well-established idiopathic mouse model of ASD known for its low sociability. We hypothesized that our conditioning paradigm would increase sociability in BTBR mice. Additionally, we wanted to examine any potential sex differences in response to the intervention, given the well-documented variability in ASD presentation between males and females. Beginning at postnatal day 21, mice were divided into four groups by sex and treatment status: male control, female control, male conditioned, and female conditioned. All mice underwent a two-day habituation period, and conditioning began on postnatal day 23. For ten days, conditioned mice were exposed to a novel conspecific and immediately received a palatable food reward (chocolate-flavored Ensure), while control mice experienced the same social interaction but received the reward two hours later, preventing an association between the stimuli. Sociability was assessed using the 3-chamber sociability assay following the 10-day

conditioning period. Social interaction levels during the conditioning period did not show any consistent patterns or learning curves across the 10 days. In the 3-chamber assay, no significant group differences emerged during the social preference phase. However, during the social novelty phase, conditioned female mice spent more time interacting with a novel conspecific than female controls. No differences were observed between male groups. These findings suggest that an ABA-style conditioning approach may enhance social novelty-seeking in female BTBR mice, highlighting the importance of sex-specific considerations when evaluating treatment outcomes in ASD and calling for replication in other ASD models.

Disclosures: B. Becher: None. **F.M. Padilla:** None. **N. Ahmed:** None. **O.L. Smith:** None. **T. Schachtman:** None. **D.Q. Beversdorf:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.04/A34

Topic: A.06. Developmental Disorders

Title: Sulforaphane Reduces Repetitive Behaviors and Enhances Social Interaction in Male and Female BTBR Mice

Authors: *O. CATALINA, S. PAPALIA, N. KERNAN, A. CRAIN, O. HOVER, S. FERNANDEZ, E. CRAIG, L. A. GABEL;
Neurosci., Lafayette Col., Easton, PA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impairments in social interaction and the presence of restricted or repetitive behaviors. Its etiology involves complex genetic and environmental interactions, with oxidative stress, marked by diminished antioxidant defenses, reactive oxygen species accumulation, and mitochondrial dysfunction, implicated as a contributing factor. The BTBR T+ Itpr3tf/J (BTBR) mouse strain is commonly used as a model for idiopathic ASD, largely based on reported deficits in social interaction and increased repetitive behaviors. Sulforaphane (SFN), a compound derived from cruciferous vegetables, has demonstrated neuroprotective potential through its antioxidant and anti-inflammatory actions. In this study, we assessed the effects of SFN (50 mg/kg, oral self-administration) on ASD-relevant behaviors in 75 mice (35 C57BL/6J [BL6]; 40 BTBR). Consistent with previous research, both male and female BTBR mice displayed elevated repetitive behavior, as measured by the marble burying test, which was attenuated by SFN treatment. Increased grooming was observed only in male BTBR mice compared to BL6 controls, with no effect of SFN. Hyperactivity was evident in both sexes relative to controls, and SFN treatment did not alter this phenotype. Anxiety-related behavior was not observed in either sex on the elevated plus maze; however, male BTBR mice displayed anxiety-like behavior on the open field test, which was exacerbated by SFN, while females did not differ from controls. Interestingly, neither male nor female BTBR mice exhibited a sociability deficit in the three-

chamber social approach task under vehicle control conditions. Nevertheless, SFN treatment increased social interaction specifically in BTBR mice of both sexes, suggesting a possible enhancement of sociability or motivation. These findings raise important questions about the sensitivity and ecological validity of the three-chamber test for detecting social impairments in BTBR mice. Given the consistent expression of repetitive behaviors but inconsistent sociability deficits, particularly under standard testing conditions, the utility of BTBR as a comprehensive model of ASD warrants re-evaluation. Future work should explore whether alternative behavioral paradigms may more effectively capture potential social deficits in this strain and further examine sex and hormonal influences on ASD-relevant behaviors.

Disclosures: O. Catalina: None. **S. Papalia:** None. **N. Kernan:** None. **A. Crain:** None. **O. Hover:** None. **S. Fernandez:** None. **E. Craig:** None. **L.A. Gabel:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.05/A35

Topic: A.06. Developmental Disorders

Support: Behavioral Neuroscience Summer Research Award

Title: Early Cannabidiol Intervention Alters Abnormal Ultrasonic Vocalization Patterns in the BTBR Mouse Model of Autism Spectrum Disorder

Authors: *S. E. MAGNOTTI[1], M. MALAN[2], R. ROBBINS[2], J. STABEN[2], J. KAPLAN[3]; [1]Psychology, [2]Western Washington Univ., Bellingham, WA; [3]Dept. of Psychology, WESTERN WASHINGTON UNIVERSITY, BELLINGHAM, WA

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by several core symptoms including social communication deficits. Recent clinical trials in individuals with ASD have found that daily treatment with a phytocannabinoid mixture rich in cannabidiol (CBD), a non-intoxicating phytocannabinoid, can improve some core and comorbid symptoms, however its impact on vocal communication deficits has not been systematically explored. The BTBR genetic mouse line has been used as a pre-clinical model for ASD due to similar behavioral phenotypes and abnormal neural signaling patterns as observed in ASD (e.g., abnormal brain derived neurotrophic factor [BDNF] levels, elevated excitatory:inhibitory signaling balance, reduced anandamide expression). Our earlier studies demonstrate prosocial effects of CBD in adult BTBR mice, but its effect on brain development and resulting behaviors in young mice is not known. We hypothesized the early developmental intervention with CBD alters brain development and rescues ASD-related symptoms. In this exploratory study, we first tested this hypothesis by assessing the impact of daily passive inhalation of vaporized CBD on hippocampal BDNF levels using Western Blot in C57BL/6J and BTBR mice during the equivalent age of adolescence. CBD normalized these levels in BTBR mice relative to the

C57BL/6J mice suggesting a powerful impact of CBD on brain development. However, even earlier symptom detection could promote earlier interventional strategies that improve outcomes later in life. To test this, we first measured USV patterns in pups at postnatal days 6, 8, and 12 during a brief period of maternal separation. BTBR mice display substantially altered USV characteristics compared to C57BL/6J mice. Daily passive inhalation of CBD vapor from postnatal days 6-12 normalized some of these characteristics by postnatal day 12 including the principal frequency, max duration, and mean peak frequency of the calls. These findings suggest that early CBD intervention can impact brain development and have potentially therapeutically relevant behavioral consequences. Future studies will assess the persistence of these effects on ASD-like behaviors in adulthood as well as seek to assess the interaction between CBD intervention, gut microbial compositions, and neurotrophic factors that may impact efficacy.

Disclosures: S.E. Magnotti: None. **M. Malan:** None. **R. Robbins:** None. **J. Staben:** None. **J. Kaplan:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.06/A36

Topic: A.06. Developmental Disorders

Support: NYS OPWDD

Title: The effects of URB507 on social, anxiety-like and motor behaviors in the BTBR mouse model of ASD

Authors: E. DEMURO[1], *K. K. CHADMAN[2];
[1]Developmental Neurobio., NYS Insitute for Basic Res. in Developmental Disabilites, Staten Island, NY; [2]Inst. for Basic Res. in Developmental Disabilities, Staten Island, NY

Abstract: Autism Spectrum Disorder (ASD), a neurodevelopmental disorder, which primarily features repetitive behaviors, restricted interests, and atypical social communication behavior. These ASD characteristics may stem from an under stimulation of the endocannabinoid system (eCB), a neuromodulatory system. Anandamide, a key endogenous endocannabinoid, is an agonist at cannabinoid receptors. URB597, a fatty acid amide hydrolase (FAAH) inhibitor, prevents the breakdown of anandamide. The BTBR T+Itpr3 tf/J (BTBR) mice, an inbred strain that naturally exhibits RRBs and atypical social behavior, serves as an idiopathic mouse model of ASD. The effects of URB597 on social and other behaviors associated with ASD were evaluated using social approach, elevated plus maze, and rotarod performance using the BTBR mice and C57BL/6J mice. Increasing anandamide with URB597 had little effect on sociability, anxiety-like behavior, or motor behavior. Previous data from our lab indicated that increasing anandamide improved repetitive behaviors in female BTBR mice. These data suggest that anandamide plays a bigger role in repetitive behaviors than social and anxiety-like behaviors.

Disclosures: E. DeMuro: None. **K.K. Chadman:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

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Topic: A.06. Developmental Disorders

Support: U0-HG007530
Sunderland Biomedical Research Fund
Engelhart Family Foundation
IDDRC-P50HD103525
Postdoctoral Fellow Seed of Independence Grant

Title: Exploring ZNF292 requirements for brain development and consequences of mutation for neurodevelopmental disorders

Authors: *K. KAUSHIK[1], M. SALEH[1], E. DANIELS-DAY[2], A. NOREN[1], J. DETERMAN[1], F. BATOOL[1], X. GE[3], G. CHAPMAN[1], S. E. MALONEY[2], J. GARBOW[3], K. K. NOGUCHI[2], K. L. KROLL[1];
[1]Dept. of Developmental Biol., [2]Dept. of Psychiatry, [3]Dept. of Radiology, Washington Univ. Sch. of Med. in St. Louis, Saint Louis, MO

Abstract: Pathogenic variants in the ZNF292 gene are a newly identified, uncharacterized contributor to neurodevelopmental disorders (NDDs). Although ZNF292 is highly expressed in both the developing and adult brain, its specific roles, mechanisms of action, and the consequences of its deficiency or mutation for neurodevelopment have remained unknown until now. To address this, we developed mouse models of ZNF292 deficiency. Our findings demonstrate that homozygous loss causes perinatal lethality, while heterozygotes (Hets) exhibit altered brain development and gene expression, as revealed by embryonic brain cortex transcriptomic analysis. Persistent ZNF292 deficiency during early gestational stages led to significant postnatal developmental impairments. Heterozygous mice (Hets) weighed less than wild-type (WTs) littermates, demonstrating compromised gross physical development. They also exhibited diminished ultrasonic vocalization calls (USV), suggesting delayed communicative behavior. Further indicators of developmental delay included a longer latency to surface right, later onset motor reflex impairment, and lower ambulation scores suggesting developmental delays. In adulthood, these heterozygous mice displayed social deficits and repetitive behaviors, such as fewer dominant bouts and increased digging and self-grooming, all consistent with NDD-relevant circuit disruptions. Parallel analysis of human stem cell models of ZNF292 pathogenic mutations revealed altered differentiation, maturation, gene expression, and function. Together, this work defines ZNF292 requirements for neurodevelopment and indicates how pathogenic mutations contribute to the etiology of NDDs.

Disclosures: K. Kaushik: None. **M. Saleh:** None. **E. Daniels-Day:** None. **A. Noren:** None. **J. Determan:** None. **F. Batool:** None. **X. Ge:** None. **G. Chapman:** None. **S.E. Maloney:** None. **J. Garbow:** None. **K.K. Noguchi:** None. **K.L. Kroll:** None.

Poster

PSTR370: Animal Models of Autism

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Topic: A.06. Developmental Disorders

Support: NIH Grant R15S088776

Title: Late-stage developmental communication in NS-PTEN mouse models

Authors: *C. LAU[1], T. BRADISH[1], S. PELL[1], M. HEMMERSMEIER[1], J. N. LUGO, Jr.[2]; [1]Baylor Univ., Waco, TX; [2]Psychology and Neurosci., Baylor Univ., Mc Gregor, TX

Abstract: Epilepsy is marked by a predisposition to experience recurrent seizures, which may arise from a variety of causes. The mechanistic target of rapamycin (mTOR) pathway is a master regulator of cell growth and proliferation. Hyperactivation of this pathway has been linked to both neurodevelopmental disorders and epilepsy. The deletion of phosphate and tensin homolog (PTEN) gene on chromosome ten—an mTOR pathway suppressor—has hyperactive mTOR signaling and is associated with the development of epilepsy. Seizures in early development have been linked to autistic-like behavioral deficits, such as communication deficits. We used a neuronal subset-specific (NS) PTEN mouse model to investigate communication differences among wildtype (WT), heterozygous (HT), and knockout (KO) genotypes. Ultrasonic vocalizations (USVs) were used to examine differences, as they can assess communication in mice. Mouse pups commonly vocalize during their first 2 weeks of life to elicit a retrieval response from the dam. We chose to examine postnatal day (PD) 14, as this represents a time of high vulnerability to seizures and marks the end of a critical period of development. We evaluated differences in genotype on call types and aspects of total calls. An analysis of the distribution of call types found that female KO had more chevron and frequency step calls and fewer complex and upward calls than female WT and HT mice ($p < .05$). Male KO were found to have more chevron, frequency step and two-component calls, and less complex, short and upward calls than WT and HT males ($p < .05$). We found that females had a greater percent of multiple calls compared to male mice ($p < .05$). Moreover, we did not find any significant genotype and sex interaction differences in USV spectral properties. We expected to find differences in the quantitative features of ultrasonic vocalizations among WT, HT, and KO NS-PTEN pups at PD14. Our results suggest that deletion of NS-PTEN, can impact critical developmental timepoints, resulting in a change in vocalization patterns. The PTEN KO mice had more changes to call type in comparison to the HT and WT mice. These findings offer deeper insights into the developmental trajectory of USVs in NS-PTEN mouse pups across genotypes.

Disclosures: C. Lau: None. **T. Bradish:** None. **S. Pell:** None. **M. Hemmersmeier:** None. **J.N. Lugo:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

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Program #/Poster #: PSTR370.09/A39

Topic: A.06. Developmental Disorders

Support: 112-2320-B-002-035-MY3

Title: Excessive repetitive behavior, enhanced spatial learning, altered dendritic features, and aberrant protein expression in Fbxo25 mutant mice

Authors: *L.-J. LEE[1], S.-Y. LIN[1], C. NG[1], H.-C. CHANG[1], S. S.-F. GAU[2];
[1]Anat. and Cell Biol., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; [2]Natl. Taiwan Univ. Hosp. & Col. of Med., Taipei, Taiwan

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by social communication impairments, restricted interests, and repetitive behaviors. A microdeletion in the 8p23.2-pter region has been identified in a Taiwanese ASD boy. The gene encoding F-box only protein 25 (FBXO25), a component of SCF (Skp1-Cullins-F-box) E3 ubiquitin ligase complexes, is located in this region. To evaluate the impact of FBXO25 removal, we established Fbxo25 knockout mice and characterized their phenotype. Both heterozygous (Het) and Homozygous (Homo) Fbxo25 knockout mice are fertile and develop normally. Compared with wild-type (WT) littermates, Fbxo25 knockout mice exhibited comparable behaviors in the aspects of locomotion, emotion, social interaction, and aggression; notably, Homo Fbxo25 displayed excessive repetitive self-grooming behavior. Given that FBXO25 is highly expressed in the hippocampus, we found reduced dendritic complexity in the granule cells of the dentate gyrus of both Het and Homo Fbxo25 knockout mice and increased dendritic spines in Het Fbxo25 knockout mice. Additionally, altered hippocampal protein expressions were noticed in knockout mice. Comparing the hippocampal protein and mRNA profiles, we identified increased GluN2A, mGluR5, ERK, and ARC proteins with unchanged mRNA expression in the Homo Fbxo25 knockout mice. Proteomic and transcriptomic profiling further identified candidate substrates of FBXO25. Gene ontology analysis highlighted pathways relevant to synaptic function, learning, and memory. Our results suggest the substrates of FBXO25-mediated protein degradation that impact the synaptic transmission and are relevant to the pathogenesis of ASD.

Disclosures: L. Lee: None. **S. Lin:** None. **C. Ng:** None. **H. Chang:** None. **S.S. Gau:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

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Program #/Poster #: PSTR370.10/A40

Topic: A.06. Developmental Disorders

Support: National Science Centre (Poland) Grant 2021/43/B/NZ5/02552

Title: Maternal high-fat diet induces sex-specific alterations in adolescent social behaviors and mPFC metabolomics in mice

Authors: *D. GAWLINSKI[1], N. MALIKOWSKA-RACIA[2], R. PIENIAZEK[1];
[1]Dept. of Drug Addiction Pharmacol., [2]Dept. of Behavioral Neurosci. & Drug Develop., Maj Inst. of Pharmacol. Polish Acad. of Sci., Krakow, Poland

Abstract: Maternal obesity, increasingly prevalent due to high-calorie diets, may significantly affect offspring neurodevelopment. Understanding these effects is crucial in light of the rising incidence of neurodevelopmental disorders (including autism spectrum disorder), suggesting a potential link between maternal metabolic status and long-term brain health in offspring. This study aimed to assess the impact of a maternal high-fat diet (HFD; 45% energy from fat) on behavioral outcomes and gut-brain axis-related metabolites in adolescent mouse offspring. Female C57BL/6J mice were fed either a control diet (CD; 10% energy from fat) or HFD for 8 weeks before mating and throughout gestation and lactation. Offspring of both sexes (n=12-15/group) were subjected to a battery of behavioral tests at developmental timepoints including ultrasonic vocalization (PNDs 4, 6, 8, 10), open field (PND 28), self-grooming (PND 33), marble burying (PND 38), reciprocal social interaction (PND 43), three-chamber social preference (PND 48), elevated plus maze (PND 53), and novel object recognition (PND 58). Additionally, we profiled gut microbiota-associated metabolites in the medial prefrontal cortex (mPFC) of offspring (n=9/group) at PND 28 using high-performance chemical isotope labeling LC-MS. Shotgun metagenomic sequencing of fecal samples revealed significantly reduced Shannon diversity in both sexes exposed to maternal HFD. Behaviorally, male HFD offspring showed a decreased number and duration of social interactions ($p<0.05$) during reciprocal interaction test, as well as impaired preference for social novelty in the three-chamber test ($p<0.05$). In contrast, female offspring from HFD group spent significantly less time in the open arms of the elevated plus maze ($p<0.05$). Moreover, metabolomic analysis identified 12 significantly altered microbiota-related metabolites in male and 14 in female mPFC samples from the HFD group, compared to control animals. These findings highlight a sex-specific vulnerability to maternal HFD exposure, with pronounced social deficits in males and anxiety-like behavior in females. Altered gut microbiota diversity and brain metabolite profiles suggest that maternal HFD may influence offspring behavior through disruptions in the gut-brain axis.

Disclosures: D. Gawlinski: None. **N. Malikowska-Racia:** None. **R. Pieniazek:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

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Program #/Poster #: PSTR370.11/A41

Topic: A.06. Developmental Disorders

Support: 112-2320-B-002-035-MY3

Title: Analysis of Repetitive Behaviors, Social Interaction, and Empathy in Dlgap2 Mutant Mice, a Mouse Model of Autism Spectrum Disorder

Authors: *Y. CHANG[1], L.-J. LEE[2], S. S.-F. GAU[3];
[1]Inst. of Anat. and Cell Biol., [2]Anat. and Cell Biol., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; [3]Natl. Taiwan Univ. Hosp. & Col. of Med., Taipei, Taiwan

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by deficits in social communication, restricted and repetitive behaviors, and impaired empathy. The prevalence of ASD has increased markedly, currently affecting 1 in 36 children, with a male-to-female ratio of 4.2:1. Despite its rising impact, the underlying pathological mechanisms remain poorly understood, necessitating the use of animal models for mechanistic and behavioral research. A clinical case of a Taiwanese boy with autism, impulsivity, and epilepsy revealed a microdeletion on chromosome 8p encompassing the Dlgap2 gene, which encodes a postsynaptic scaffolding protein essential for synaptic signaling and organization. This study aims to evaluate core ASD-related phenotypes in Dlgap2 mutant mice, including repetitive behaviors, social interactions, and empathy. Locomotor activity was intact across genotypes, while Dlgap2 heterozygous (Het) mice exhibited increased anxiety levels. Behaviorally, Het mice showed reduced rearing, and knockout (KO) mice displayed significantly less self-grooming with shorter bout durations and fewer transitions per bout. Detailed grooming microstructure analysis further revealed that both Het and KO mice exhibited abnormalities in grooming phases 4 and 5, suggesting a possible impairment in perceiving grooming completion. In the social interaction test, KO mice showed increased sociability, although the underlying mechanisms require further investigation. In the Witnessing Cagemate in Pain (WCP) test, wild-type (WT) mice demonstrated empathic behavior by comforting distressed cage mates, a response notably absent in Het and KO mice—consistent with the clinical phenotype observed in the patients. This research underscores the role of Dlgap2 in ASD-related behaviors and provides novel insights into behavioral phenotyping strategies for ASD mouse models, with implications for future diagnostic and therapeutic developments.

Disclosures: Y. Chang: None. **L. Lee:** None. **S.S. Gau:** None.

Poster

PSTR370: Animal Models of Autism

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Program #/Poster #: PSTR370.12/A42

Topic: A.06. Developmental Disorders

Support: NCN 2022/45/B/NZ4/03262

Title: Lipocalin-2 Deletion and Maternal Immune Activation Converge on Common Behavioral and Transcriptional Outcomes in the Developing Mouse Brain

Authors: M. PEKALA, S. ZAWISLAK, L. KACZMAREK, ***K. KALITA**, K. NADER; Nencki Inst., Warsaw, Poland

Abstract: Maternal infection during pregnancy is a well-established risk factor for neurodevelopmental disorders (NDDs), yet the underlying molecular mechanisms remain poorly understood. Lipocalin-2 (Lcn2), an innate immune protein that is highly upregulated during infection, also affects neuronal and glial function. This study investigates the role of Lcn2 in shaping neuronal development, particularly after maternal immune activation (MIA). To mimic maternal infection, pregnant mice received intraperitoneal injections of lipopolysaccharide (LPS) or saline on embryonic days 16 to 18 to model the second trimester of pregnancy in humans. We first confirmed that Lcn2 is expressed in the fetal brain and that MIA significantly upregulates Lcn2 mRNA and protein in the hippocampus and cortex from both sexes. To assess functional relevance, we utilized Lcn2 heterozygous breeding to generate wild-type and Lcn2-knockout offspring from the MIA and control groups. Both female and male offspring underwent a battery of behavioral assays. Lcn2 deletion and MIA independently induced deficits in social behavior and increased repetitive behavior phenotypes relevant to NDDs. However, their combination did not exacerbate these effects, suggesting an occlusion effect. To investigate potential shared molecular mechanisms, we performed RNA sequencing of the fetal forebrain 4 hours after the final LPS injection. This analysis revealed a common set of differentially expressed genes in both the Lcn2-knockout and MIA groups, indicating convergence on similar transcriptional pathways that may underlie the observed behavioral phenotypes. These results suggest that while Lcn2 may not mediate the pathological effects of prenatal immune challenge, it plays a critical role in normal brain development.

Disclosures: M. Pekala: None. **S. Zawislak:** None. **L. Kaczmarek:** None. **K. Kalita:** None. **K. Nader:** None.

Poster

PSTR370: Animal Models of Autism

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Program #/Poster #: PSTR370.13/A43

Topic: A.06. Developmental Disorders

Support: National Science Centre (Poland) Grant 2023/51/D/NZ5/02090

Title: Maternal high-fat diet alters dendritic spine density of mouse offspring cortical neurons: potential modulation by short-chain fatty acids

Authors: K. GAWLINSKA[1], A. WESOLOWSKA[2];
[1]Dept. of Clin. Pharm., Jagiellonian Univ. Med. Col., Krakow, Poland; [2]Dept. of Clin. Pharm., Jagiellonian Univ. Med. Col., Kraków, Poland

Abstract: Maternal nutrition during gestation has a profound impact on offspring neurodevelopment. Emerging evidence implicates a maternal high-fat diet (HFD) in altering brain structure and function, potentially via the gut-brain axis and microbial metabolites such as short-chain fatty acids (SCFAs). Altered dendritic spine density is a hallmark of disrupted synaptogenesis, associated with neurodevelopmental disorders, including autism spectrum disorder (ASD). Here, we examined whether maternal HFD affects dendritic spine density in cortical neurons of mouse offspring and whether SCFAs can modulate these effects.To investigate the impact of maternal diet on offspring brain development, we utilized primary cortical neuron cultures derived from embryonic day 18.5 (E18.5) offspring of C57BL/6J mouse dams. Before mating and throughout gestation, females were fed either a control diet (CD; 10% kcal from fat) or a high-fat diet (HFD; 45% kcal from fat). Cortical neurons were cultured and stained with the lipophilic dye DiI to visualize dendritic spines. Spine density was quantified using confocal microscopy and SpineJ software. To further explore potential modulatory effects of microbial metabolites, neurons from HFD offspring were treated on day in vitro 18 (DIV18) with short-chain fatty acids (SCFAs) - sodium acetate (SA, 1 mM), sodium butyrate (SB, 1 mM), sodium propionate (SP, 1 mM), or a mix of all SCFAs (1 mM each) - for 48 hours. Quantitative analysis revealed a significant increase ($p < 0.01$) in dendritic spine density in cortical neurons derived from HFD-exposed offspring compared to the CD group, indicating altered synaptic development. Preliminary data suggest that treatment with SB showed a tendency toward normalization of spine density in the HFD group. These findings suggest that maternal HFD induces persistent changes in neuronal morphology in the offspring, potentially mediated via the gut-brain axis.

Disclosures: K. Gawlinska: None. **A. Wesolowska:** None.

Poster

PSTR370: Animal Models of Autism

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Topic: A.06. Developmental Disorders

Support: JSPS KAKENHI 23KJ1519

Title: Cortical hyperactivity underlies impaired social interaction in a mouse model of 3q29 microdeletion

Authors: *T. TAKEMOTO[1], K. KITAGAWA[1], K. NAGAYASU[1], K. SEIRIKI[1], A. KASAI[2], Y. AGO[4], K. TAKUMA[5], D. MORI[6,3,7], N. OZAKI[7,8], R. HASHIMOTO[9], H. HASHIMOTO[1,9,10,11,12], T. NAKAZAWA[1,9,13];
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Abstract: The 3q29 microdeletion, a recurrent copy number variant, is strongly associated with an increased risk of both schizophrenia and autism spectrum disorders, providing a valuable pathological model for investigating the underlying mechanisms of psychiatric conditions. Previously, we generated a mouse model carrying a deletion in the mouse orthologous region of the human 3q29 locus (Df/+ mice). Df/+ mice exhibit behavioral abnormalities relevant to psychiatric symptoms, including impaired social interaction, providing a valuable model for psychiatric conditions. Notably, Df/+ mice display cortical hyperactivity in response to social stimuli, suggesting a potential link between cortical hyperexcitability and impaired social interaction. To assess the pathogenic role of this cortical hyperactivity in Df/+ mice, we examined social behavior in mice by chemogenetic modulation of cortical activity. We found that suppression of neural ensembles responsive to social stimuli markedly rescued impaired social behavior in Df/+ mice. Conversely, chemogenetic activation of excitatory neurons significantly decreased social behavior in wild-type mice, without affecting locomotion or anxiety-like behavior. These findings suggest that the cortical hyperactivity contributes to impaired social interaction in this mouse model, and that targeting the hyperactivity may be a therapeutic strategy for restoring social function. Our results provide new insights into neurological pathogenesis associated with the 3q29 microdeletion.

Disclosures: T. Takemoto: None. **K. Kitagawa:** None. **K. Nagayasu:** None. **K. Seiriki:** None. **A. Kasai:** None. **Y. Ago:** None. **K. Takuma:** None. **D. Mori:** None. **N. Ozaki:** None. **R. Hashimoto:** None. **H. Hashimoto:** None. **T. Nakazawa:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.15/A45

Topic: A.06. Developmental Disorders

Support: Scobie Family
Bristol Neuroscience

Title: Cerebellar influence on prefrontal circuits supporting social behaviour in a model of autism spectrum disorder

Authors: *D. MEDIANE;
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Abstract: Social behavioural deficits are a core feature of autism spectrum disorder (ASD), yet the neural mechanisms underlying these behavioural traits remain poorly understood. Recent work has highlighted the cerebellum's role not only in motor coordination but also in shaping higher-order functions, including those related to affective and social domains. Increasing evidence suggests that cerebellar outputs influence activity in the medial prefrontal cortex (mPFC) and may contribute to some of the atypical behavioural patterns observed in ASD. However, the nature of this influence and its relevance to social functioning is not fully understood. To investigate how brain circuits contribute to social behaviour differences observed in ASD, we use an established mouse model that displays autism-like traits (PCP2-Cre;Tsc1flox/flox), alongside litter-mate controls. This model allows us to explore how altered cerebellar function might influence the mPFC. To resolve this question, we adopt a systems and circuit level perspective, including cFOS staining, anatomical tracing and chemogenetics, to investigate how interactions between the cerebellum and mPFC govern social behaviour. The goal is to contribute to a growing understanding of how distributed brain networks give rise to behavioural variation relevant to neurodevelopmental conditions. By characterising features of this ASD-relevant model, we aim to inform broader efforts to identify circuit-level mechanisms that support typical and atypical social functions.

Disclosures: D. Mediane: None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.16/Web Only

Topic: A.06. Developmental Disorders

Title: Evaluation of dendritic spine density in level IV primary somatosensory cortex using custom diolistic labeling in a developmental hyperserotonemia rat model for autism spectrum disorder

Authors: *L. HOUGH, L. STAUDT;
Kansas City Univ., Joplin, MO

Abstract: Developmental hyperserotonemia (DHS) is the most consistent neurochemical finding reported in autism and has been implicated in the pathogenesis of ASD. Accordingly, it has been hypothesized that pre-and postnatal administration of the non-selective serotonin agonist, 5-methoxytptamine may serve as a model to examine behavioral and cytoarchitectural characteristics associated with ASD. Our previous investigations of the DHS model have demonstrated significant neurodevelopmental changes in the architecture, connectivity, and function of various areas of the motor and somatosensory system. ASD is associated with deficits in social cognition, disordered communication, restricted interests and repetitive behaviors, abnormalities in basic motor control, skilled motor gestures, and motor learning. These motor and somatosensory features may be linked to possible disruptions in the pre- and postnatal development of specific neural networks including the dentate-thalamo-cortical pathway, and the primary somatosensory cortex. This study presents a method to further analyze the morphology and connectivity of cortical neurons located in level IV of the primary somatosensory cortex of DHS animals. A modified diolistic labeling protocol was developed utilizing a custom laboratory-built gene gun to label fixed tissue sections with lipophilic carbocyanine dyes. This method allowed for cost effective, reproducible visualization and analysis of cortical neuronal morphology. The use of lipophilic carbocyanine dyes, combined with particle- mediated biolistic delivery facilitates non-toxic fluorescent labeling of multiple neurons and dendritic arbors in fixed tissue. Confocal microscopy and 3-dimensional neuronal tracings were performed to evaluate neuronal morphology in the DHS and control populations.

Disclosures: L. Hough: None. **L. Staudt:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.17/A46

Topic: A.06. Developmental Disorders

Title: S-adenosylmethionine modulates autism risk and redox potential genes expression changes induced by prenatal valproic acid exposure in fetal mice brains

Authors: *B. E. ECHEFU[1], M. BECKER[2], A. ORNOY[3,1];
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Abstract: Valproic acid (VPA) is a potent teratogen and neurotoxicant. Prenatal VPA administration in mice induces exencephaly and/or autistic like behavior (ASD). Concomitant administration of S-adenosylmethionine (SAMe) prevented these changes. In this study we investigated the outcome of VPA and SAMe coadministration during neurulation on the

expression of genes related to autism risk and oxidative and nitrosative stress defense in the brain 7 days after exposure. Pregnant mice received intraperitoneally during gestation days (GD) 8 and 9 either normal saline (controls) or 300 mg/kg VPA, or 30 mg/kg SAMe or the combination of VPA and SAMe, which was administered one hour before VPA. Another group was treated with a mixture of VPA and SAMe. On GD 15 fetal brains were collected. Brains (exhibiting exencephaly in the VPA - treated fetuses were studied separately). Real time PCR was performed on the brains using primers for selected genes. We report that *in utero* VPA administration during neurulation induced changes in autism risk genes that persisted throughout fetal development, and in genes associated with oxidative and nitrosative stress defense. VPA induced in the whole brain increase in the expressions of Notch, IGF1, VEGFA, MECP2 and significant down regulation of OXTR. VPA also induced upregulation in the expression of antioxidant genes SOD1, SOD2, GPX1, CAT, NOS1 and NOS2. SAMe pre-treatment, or combined treatment with VPA, reversed (normalized) the effects of VPA alone on most of these genes. We presume that the VPA - induced changes in gene expression contribute, at least partially, to NTD and/or ASD like behavior. SAMe, which is also affecting the epigenome, seems to have the potential to correct the gene expression changes induced by VPA, thus preventing both of VPA-induced NTD and ASD like behaviors.

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

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.18/A47

Topic: A.06. Developmental Disorders

Support: Genetic Autism Alliance

Title: Constructing a mouse model of synaptotagmin-1 point mutation

Authors: *A. RAJOOLDEZFULY[1], Z. WANG[2], A. SAFAEI[5], B. YU[2], W. ARNOLD[3], D. DAVIS[5], D.-P. LI[6], P. R. CARNEY[4];
[1]Univ. Of Missouri, Columbia, Columbia, MO; [3]NextGen Precision Hlth., [4]Child Hlth., [2]Univ. of Missouri, Columbia, Columbia, MO; [5]Univ. of Missouri-Columbia (SSO), Columbia, MO; [6]Med., Univ. of Missouri, Columbia, MO

Abstract: Constructing a mouse model of Synaptotagmin-1 point mutation Ali Rajooldezfuly, Zhonglei Wang, Azadeh Safaei, Baocong Yu, David Arnold, Daniel Davis, De-Pei Li, Paul R. Carney,University of Missouri, Columbia Synaptotagmin-1 (Syt1), a presynaptic calcium sensor, is essential for synchronous neurotransmitter release and regulates spontaneous neurotransmitter release. Mutations in the C2B domain of Syt1 disrupt Ca^{2+}-dependent exocytosis result in Syt1-associated neurodevelopmental disorder (Syt1-NDD)—a severe condition characterized by cognitive impairment, motor deficits, and social behavior abnormalities. No effective therapies

exist, and mechanistic insights remain unclear. The objective of our study was to establish a novel AAV-mediated mouse model expressing a pathogenic Syt1 mutation (D366E) in the hippocampus and evaluate behavioral and synaptic alterations.C57BL/6 mice were received hippocampal and cortical (prelimbic and infralimbic regions) injections of AAV9-CBA-Syt1(D366E)-P2A-EGFP-WPRE, AAV9-CBA-WT-Syt1-P2A-mCherry-WPRE, and AAV9-CBA-EGFP-WPRE. Behavioral assays were performed to assess their activity, social interaction, anxiety levels, and cognitive function. Spontaneous and evoked excitatory postsynaptic currents (EPSCs) were recorded from hippocampal CA1 neurons in brain slice using whole-cell patch-clamp approach to assess neurotransmitter release.Mice expressing mutant Syt1 displayed impaired sociability and social novelty in the 3-chamber test, indicative of autism-like behaviors. In contrast, anxiety, locomotion, and recognition memory were not significantly different from AAV-eGFP injected mice. The frequency of spontaneous EPSCs increased while their amplitude was decreased in mutant Syt1 mice compared to eGFP expressing mice. Furthermore, the amplitude of evoked EPSC recorded from CA1 pyramidal neurons were significantly reduced in mutant Syt1 mice compared to eGFP expressing mice. These data suggest that mutant Syt1 disrupts synchronized glutamatergic synaptic transmission.This AAV-mediated mutant-Syt1 hippocampal model recapitulates key behavioral and synaptic phenotypes associated with Syt1-NDD. This mouse model is a valuable platform for elucidating disease mechanisms and evaluating targeted therapeutic strategies for Syt1-NDD. Keywords: Synaptotagmin-1; synaptic transmission; Neurodevelopmental disease

Disclosures: **A. Rajooldezfuly:** A. Employment/Salary (full or part-time): University of Missouri, Columbia. **Z. Wang:** A. Employment/Salary (full or part-time): University of Missouri. **A. Safaei:** A. Employment/Salary (full or part-time): University of Missouri. **B. Yu:** A. Employment/Salary (full or part-time): University of Missouri. **W. Arnold:** A. Employment/Salary (full or part-time): University of Missouri. **D. Davis:** A. Employment/Salary (full or part-time): University of Missouri. **D. Li:** A. Employment/Salary (full or part-time): University of Missouri. **P.R. Carney:** A. Employment/Salary (full or part-time): University of Missouri.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.19/A48

Topic: A.06. Developmental Disorders

Support: NIH Grant ES025585
NIH Grant MH112763

Title: Delayed Epigenetic Programming and Altered Physiological Maturation of Cortical Excitatory Neurons Following Maternal Immune Activation

Authors: J. ARZAVALA[1,4], C.-Y. LAI[2], A. PINTO-DUARTE[2], J. LI[5], H. LIU[3], J. OSTEEN[2], S. B. POWELL[6], J. R. ECKER[3], E. A. MUKAMEL[5], M. BEHRENS[2,6];
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Abstract: Viral infection during pregnancy has been linked to altered neurodevelopment in offspring, increasing the risk of disorders such as schizophrenia and autism spectrum disorder. Pro-inflammatory cytokines released in response to maternal immune activation (MIA) are thought to mediate these effects on fetal brain development. Animal models of MIA recapitulate behaviors resembling neurodevelopmental disorder phenotypes, but genomic differences in adult offspring are often subtle. This suggests that the long-term effects of MIA may originate from early disruptions in neuronal and circuit formation. We investigated transcriptional, epigenomic, and electrophysiological alterations in perinatal excitatory cortical neurons following MIA. By focusing on the perinatal period—from late embryonic stages to the first two postnatal weeks—we aimed to understand how MIA impacts the transition from embryonic to mature programming in a major neuronal class of the frontal cortex. Using an INTACT mouse line (Mo et al., 2015) to label excitatory neurons, Poly(I:C) was administered to pregnant dams at embryonic day (E) 12.5 to simulate a severe inflammatory reaction during mid-gestation. Fluorescently activated nuclei sorting (FANS) was used to isolate the excitatory population from the frontal cortex of male offspring for RNA and whole-genome bisulfite sequencing. We found early disruption of progenitor cell proliferation genes at E15, followed by stalled synaptic development at birth, marked by dysregulated genes associated with scaffolding proteins, glutamatergic transmission, and neurotransmitter receptors. Furthermore, genes predominantly expressed in deep-layer cortical neurons were disrupted at P0. MIA significantly altered the developmental timing of DNA methylation patterns. Genomic regions that typically gain methylation by birth remained unmethylated in Poly(I:C) offspring, while regions that normally lose methylation were hypermethylated. In addition, patch-clamp recordings revealed that pyramidal neurons in layer 5—but not in layer 2/3—exhibited electrophysiological properties indicative of immature intrinsic function. Together, these findings suggest that MIA causes aberrant epigenomic timing and disrupts physiological maturation in developing excitatory neurons in the frontal cortex. Moreover, our results suggest that MIA during mid-gestation preferentially impairs deep-layer cortical excitatory neuronal development. This work offers new insights into how prenatal inflammation may impair early circuit formation and cell-type-specific trajectories across cortical layers.

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Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: A.06. Developmental Disorders

Support: NIH NIMH R01MH113926
NIH NCATS TL1TR002371
OHSU Richard & Marilyn Jones Endowed Fund for Advancement in Autism Research
OHSU Brain Institute Neuroscience Campaign Fund to Support Brain Health Across the Lifespan
Medical Research Foundation
Collins Medical Trust

Title: Defining mechanisms of TBR1 Syndrome using diverse allelic variants

Authors: *M. CO[1], D. WEINGARTEN[2], G. O'BRIEN[3], K. PATTON[3], S. JACKMAN[2], K. WRIGHT[2], B. J. O'ROAK[3];
[2]Vollum Inst., [3]Mol. and Med. Genet., [1]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: TBR1 Syndrome is a rare form of autism and intellectual disability caused by heterozygous *de novo* variants in *TBR1*. *TBR1* encodes a transcription factor required in the developing cortex for proper layer formation, neuronal subtype specification, and axon development. Despite harboring variants within the same gene, individuals with TBR1 Syndrome exhibit variability in clinical symptoms, severity, and structural MRI findings. The distribution of *TBR1* variants predicts at least 3 functional classes potentially contributing to this variability: (1) early-truncating variants leading to haploinsufficiency, (2) missense variants impacting the T-box DNA-binding domain, and (3) late-truncating variants impacting the C-terminal domain. Late-truncating variants, including the highly recurrent frameshift p.T532Rfs*144, are associated with more severe clinical phenotypes and cortical malformations. We hypothesize that *TBR1* variant classes contribute to clinical heterogeneity by differentially impacting TBR1 protein levels and functions. Using CRISPR, we generated an allelic series of mice representing the three predicted *TBR1* variant classes. Heterozygotes of each class demonstrate face validity for TBR1 Syndrome by phenocopying anterior commissure reductions observed in patient MRIs. We observe differential impacts of variants on TBR1 protein levels in postnatal cortex: early-truncating variants decrease TBR1, missense variant p.K228E increase TBR1, and late-truncating frameshift variants decrease or increase TBR1 depending on the mutant reading frame generated. We also observe differential impacts of variants on cortical layering, indicating functional differences among alleles. Using RNA-seq, we identify shared and unique differentially expressed genes between early-truncating and missense mutants, with extracellular matrix-, neurotrophin-, and axon-related pathways represented among shared genes. We also identify shared and unique electrophysiological properties of early-truncating and missense projection neurons, including altered NMDA/AMPA ratios in mPFC. Using behavioral assays, we observe impaired postnatal ambulatory function and altered social behavior, with preliminary differences by variant. Finally, our preliminary biochemical assays indicate differences in protein stability and protein-protein interactions among missense and late-truncating variants. Altogether, using a comprehensive allelic toolkit, we provide evidence for variant-specific mechanisms that may contribute to TBR1 Syndrome heterogeneity and inform tailored treatment approaches.

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Poster

PSTR370: Animal Models of Autism

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Topic: A.06. Developmental Disorders

Support: NIH Grant NS111760
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FOXG1 Research Foundation
Simons Foundation SFARI 1013720
University at Buffalo

Title: Striatal hypoplasia and SPN deficits in a Q84Pfs/+ mouse model of FOXG1 Syndrome

Authors: *V. DUVVURU[1], S. JEON[2], S.-K. LEE[1], J. LEE[1];
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Abstract: Forkhead Box G1 (FOXG1) is a winged-helix transcription factor that is considered a pivotal regulator of telencephalic development, with expression initiating during early stages of embryonic development in telencephalic neuroepithelial progenitors. In addition to its well-established roles in early neurogenesis, including the regulation of neuronal proliferation, migration, and differentiation, FOXG1 remains actively expressed in postmitotic neurons of the cortex, hippocampus, and striatum throughout postnatal and adult life. In humans, homozygous *FOXG1* mutations are embryonically lethal, while *de novo* heterozygous mutations lead to FOXG1 Syndrome (FS). This severe neurodevelopmental disorder is characterized by microcephaly, epilepsy, malformations of the corpus callosum, delayed myelination, and broad motor, cognitive, and social deficits that often resemble symptoms of autism spectrum disorder (ASD). One such mutation, Q86Pfs/+, introduces a frameshift that produces a truncated protein retaining only the N-terminal region, resulting in haploinsufficiency and loss of FOXG1 function. To investigate the contribution of this mutation to disease pathology, we examined the Q84Pfs/+ mouse model, which is homologous to the human Q86Pfs/+ variant. Given the striatum's role in integrating motor, cognitive, and affective functions disrupted in FS, we centered our analysis on striatal development. Using a multimodal approach, we assessed relative striatal volume as an indicator of potential hypoplasia and used histological studies to evaluate the expression of canonical striatal projection neuron (SPN) markers to identify cellular abnormalities in SPN development. We profiled the striatum at three key neurodevelopmental stages: embryonic day 15.5 (E15.5), postnatal day 0 (P0), and postnatal day 9 (P9), which

correspond to peak SPN proliferation, differentiation, and early functional maturation, respectively. Our developmental profile aims to uncover structural and cellular abnormalities in the striatum, with a focus on hypoplasia and alterations in SPN lineage marker expression associated with Foxg1 haploinsufficiency (n=3). Given the limited prior characterization of striatal development in the context of Foxg1 haploinsufficiency, this study adopts an exploratory approach. Our objective is to characterize structural and cellular features contributing to early striatal abnormalities in a clinically relevant FS model. These findings may ultimately inform biomarkers for disease progression and identify potential targets for therapeutic intervention.

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Poster

PSTR370: Animal Models of Autism

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Topic: A.06. Developmental Disorders

Support: Jordan's Guardian Angels charity, JGA grant to JLS
UC Davis MIND Institute Intellectual and Developmental Disabilities Research Center (IDDRC) discretionary fund (NIH/NICDH grant #P50HD103526 to L. Abbeduto, JLS)

Title: Mice carrying the pathogenic E198K mutation of the PPP2R5D gene exhibit developmental deficits reminiscent of Jordan's Syndrome phenotypes

Authors: *A. Y. YOTOVA[1], A. J. ALIDAEE[1], S. STRACK[2], J. L. SILVERMAN[1];
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Abstract: Jordan's Syndrome (JS), also known as PPP2R5D-related intellectual disability, is a rare neurodevelopmental disorder characterized by mutations in a regulatory part of the protein phosphatase 2A (PP2A), which is highly expressed in the brain and implicated in its proper function via regulation of multiple downstream cellular signaling pathways. JS patients exhibit high incidence of developmental delays, cognitive deficits, autism spectrum disorder, motor impairments, macroencephaly, and seizure susceptibility, among others. This study utilizes mice (on *C57BL6/J* background) carrying the E198K mutation in the regulatory subunit of PP2A, which represents an amino acid swap that affects its phosphatase binding abilities with deleterious effects. The mutation reportedly corresponds to more severe phenotypes in JS patients and is lethal in mice when present in both gene copies. Thus, we utilized heterozygous carrier mice and littermates lacking the mutation. Developmental milestone analyses (n=10-12 mice/genotype, from six litters) were performed on minimum of nine days between birth and postnatal day 17 and revealed multiple developmental delays in the mutant mice. These included significantly lower body weight and length, as well as deficits in mobility, righting reflex, and

grip strength, which mimic relevant phenotypes of children with JS. Additionally, we observed abnormal domed skull and curved muzzle shape, which are in line with dysmorphic facial features and macroencephaly frequently observed in JS patients.As adults, the mutant mice of both sexes exhibited reduced vertical activity in the open field test of spontaneous locomotion. Ongoing experiments implement automated gait analysis and rotarod test in adults to parse through motor endophenotypes resulting from the E198K mutation. We are also currently investigating the neurophysiological markers of E198K mutant mice using wireless telemeters to measure electroencephalographic (EEG) signatures. These would provide important translational value as a majority of E198K JS patients suffer from seizures and pathological changes in multiple EEG facets have been reported. Taken together, our findings and ongoing testing of E198K mice should provide further insights into mutation-specific deficits related to JS and opportunities to test compounds aimed at alleviating deleterious phenotypes.

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Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.23/Web Only

Topic: A.06. Developmental Disorders

Support: 22K07611

Title: Temporal and spatial mapping of IL-17 receptor expression in the cerebral cortex: Analysis using an ASD model

Authors: *T. SASAKI[1], A. KUBO[2], S. KAMIYA[2], K. NAKAMURA[3], S. SANAKA[2], K. KISHI[2], S. IWATA[2], Y. TAKEI[2];
[2]Inst. of Med., [3]Fac. of Med., [1]Univ. of Tsukuba, Tsukuba, Japan

Abstract: Recent studies suggest that immune dysregulation, especially involving T helper 17 (Th17) cells, contributes to psychiatric disorders such as autism spectrum disorder (ASD), schizophrenia, and depression. IL-17A, produced by Th17 cells, signals through a receptor complex of IL-17RA and IL-17RC, activating inflammatory pathways. In maternal immune activation (MIA), maternal infections trigger IL-17A production, which may cross the placenta and affect fetal brain development, contributing to ASD (Choi et al., 2016; Kubo et al., 2024). Although IL-17 receptor expression in the brain has been reported, its distribution and functional relevance remain unclear. To investigate this, we examined the developmental expression of *Il17ra* and *Il17rc* mRNA in mouse brains using in situ hybridization, and IL-17RA protein localization via immunostaining. We also assessed changes in receptor expression in the cortex of MIA model mice. *Il17ra* was strongly expressed in cortical layers V-VI from postnatal day 7 to 14 and declined with age. *Il17rc* was broadly distributed across layers II-VI from embryonic to early postnatal stages. In adults, *Il17ra* showed region-specific expression in sensory cortex,

while *Il17rc* remained diffusely expressed. IL-17RA was found in DCX-positive immature neurons during development and in TBR1-positive layer VI corticothalamic neurons postnatally. Neither receptor was detected in astrocytes or microglia. In MIA mice, *Il17rc* expression was unchanged, but *Il17ra* was significantly reduced at P14, suggesting a link to ASD-related circuit alterations. These findings indicate that IL-17A receptors are primarily expressed in neurons during brain development and may mediate immune-related influences on cortical formation. Future studies will test how direct fetal exposure to IL-17A impacts cortical structure.

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Poster

PSTR370: Animal Models of Autism

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Program #/Poster #: PSTR370.24/A52

Topic: A.06. Developmental Disorders

Support: ARI Pilot Grant

Title: Mirna as a potential mediator in maternal/fetal interaction in neurodevelopmental disorders

Authors: *N. AHMED[1], O. L. SMITH[2], H. E. STEVENS[5], M. J. WILL[3], D. Q. BEVERSDORF[4];
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Abstract: The Developmental Origins of Health and Disease hypothesis (DoHaD) purposes that environmental stress exposure to the intrauterine and maternal systems at key developmental periods may lead to long-term impacts on the health of the offspring. Our previous mouse model study demonstrated that the combination of maternal SERT heterozygosity and prenatal stress exposure resulted in reduced social preference and increased repetitive behaviors in male offspring, compared to wildtype controls. Additionally, we identified three differentially expressed maternal miRNAs (mmu-miR-7684-3p, mmu-miR-5622-3p, mmu-miR-6900-3p) in the SERT-het/stress group demonstrating the epigenetic changes in the miRNA profile at the time of pregnancy. To investigate the mechanistic role of the prenatal stress-associated miRNAs, they were replicated as oligonucleotide miRNA mimics and combined into a miRNA mix diluted to 50 ug/100 uL. C57Bl/6J pregnant mice were then injected with 100 uL on gestational day 6 (G6) and again on G14. Starting from post-natal day 60, offspring from injected and injection naive mice were behaviorally tested for social communication, repetitive behaviors, and other anxiety measures. Offspring displayed no differences in social interactions or repetitive grooming behaviors but, during open field testing, 18/23 (80%) of tested offspring from injected

dams displayed spontaneous, and repetitive jumping directly into the wall of the chamber. They exhibited decreased duration within the center of the open field and this was corroborated by higher thigmotaxis. However, prenatally miRNA exposed offspring also displayed increased distance travelled during the elevated plus maze as well as longer duration spent on open arms than non-injected controls, suggesting differing anxiety-like phenotypes. These findings suggest maternal intravenous exposure to the three prenatal stress-related miRNA recapitulates specific behavioral patterns of hyperlocomotion and wall-directed repetitive behaviors independent of social ability and self-grooming behaviors.

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Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.25/A53

Topic: A.06. Developmental Disorders

Support: CLIN1 (Silverman) CLIN1-17103, Expression of Ube3a by the hematopoietic system for the treatment of Angelman syndrome
R01 (Silverman) Project Number: 1R01NS097808-A1 Phenotypic Characterization of Novel Models of Dup15q Syndrome
P50 (Abeddutto) P50HD103526

Title: Neurophysiological phenotypes and translational biomarkers in a novel mouse model for Dup15q syndrome

Authors: *E. R. MONSEN[1], A. Y. YOTOVA[3], O. HAOUCHINE[4], J. R. LASALLE[2], D. YASUI[5], J. L. SILVERMAN[6];
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Abstract: UBE3A is a tightly controlled, gene, that is epigenetically regulated, within human chromosome 15q11.2-q13.3. It is paternally imprinted thus expression is exclusively from the maternal allele. *De novo* mutations inherited from the maternal allele in this region/gene result in rare genetic neurodevelopmental disorders (NDD), from either the loss of or over excess of Ube3a expression, termed, Angelman and Dup15q Syndromes, respectively. Phenotypically, both ASDs, often have a co-occurring autism spectrum disorder (ASD) diagnosis. However, while they share some characteristic traits such as developmental delay and intellectual disabilities, there clinical phenotypes are distinct. Seizures exist in both AS (25-30%) and

Dup15q (>70%), however, they also phenotypically differ in severity. In Dup15q, recurring, persistent, treatment resistant epilepsy often results in Sudden Unexpected Death due to Epilepsy (SUDEP). Our laboratory has been researching these disorders, for nearly a decade, we have shown that the loss of *Ube3a* greatly affected the neurophysiological presentation with elevated spiking, elevated delta spectral power and numerous anomalies in sleep (Copping et al., 2021). We also have illustrated data on our newly constructed, piggyBac-on-BAC transgenic experimental model, with a clinically relevant level of Ube3a overexpression (OE), with different two levels of Ube3a-overexpression, modelling the two etiologies of Dup15q. Therefore, we hypothesize that *UBE3A* overexpression (OE; 1X, 2X) is the causal factor in dysfunctional Dup15q neurophysiology in seizures, spiking, spectral power densities, and sleep. Preliminary data illustrate hyperexcitability, excess spike trains, differential power spectral density and poor slow wave sleep. Clinical AS EEG data cand preclinical AS data collected in the standard *Ube3a-del* $^{m-/p+}$ mouse model of AS illustrated elevated delta spectral power and reduced beta oscillations. Increased beta spectral power, has been reported clinically a proposed biomarker of Dup15q Syndrome. Our goal is to identify non behavioral, objective translational biomarkers to help to better predict severity of outcomes, monitor treatment responsivity, reduce genotypic and phenotypic heterogeneity, and improve the preclinical to clinical translational pipeline. Our novel *Ube3a*-tg OE 1X and 2X models provide critical tools and key phenotypic discoveries to uncover disrupted neural circuits in Dup15q and related genetic NDDs, to identify malleable biomarkers, and explore therapeutic interventions.

Disclosures: E.R. Monsen: None. **A.Y. Yotova:** None. **O. Haouchine:** None. **J.R. LaSalle:** None. **D. Yasui:** None. **J.L. Silverman:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.26/A54

Topic: A.06. Developmental Disorders

Support: R01MH120513
R21MH132834
T32MH112507

Title: Multimodal study of the molecular consequences of CHD8 haploinsuffiencey on mouse brain development and persistence into adulthood

Authors: *N. SEBAN[1], C. P. CANALES[1], M. TABBAA[2], S. A. LOZANO[1], K. CICHEWICZ[1], E. FENTON[1], N. CHU[1], S. JUNG[1], D. RAHBARIAN[1], A. KOPLEY-SMITH[1], D. FIORAVANTE[1], A. S. NORD[1];
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Abstract: Mutations in the chromatin-remodeling factor *CHD8* are strongly associated with autism spectrum disorder (ASD). *Chd8* haploinsufficient mice display genomic, neuroanatomical, and behavioral abnormalities that are relevant to ASD and phenotypes in human *CHD8* mutation carriers. However, reported neurodevelopmental phenotypes vary across mouse studies. We sought to identify conserved and distinct perturbations in forebrain development caused by *Chd8* haploinsufficiency in distinct loss-of-function mouse models. Our analysis encompassed pallial and subpallial cell populations at embryonic (E14.5) and early postnatal (P2) stages using single-cell multiomic (RNA-seq and ATAC-seq) approaches. We assessed cell-type-specific differences in abundance, developmental rate, differential expression, and differential accessibility. Additionally, we compared bulk RNA-seq transcriptional profiles across multiple genetic backgrounds and the same Chd8 mouse model to explore broader variability during neurogenesis. Finally, we extended analysis to test if pathology identified in the developing forebrain persisted into adulthood or was conserved in other brain regions. Our findings reveal that there is a subtle transient imbalance to pallial (excitatory) vs subpallial (inhibitory) neurogenesis at E14.5, with gliogenesis more stongly impacted at P2. We identified disrupted transcriptional and epigenetic programs in specific telencephalic lineages and cell types at both E14.5 and P2. Bulk RNA-seq data at E14.5 showed significant variation across genetic backgrounds, particularly in terms of proliferation and neuronal maturation. By integrating multiple models and genetic backgrounds, we identified conserved molecular pathologies associated with *Chd8* haploinsufficiency during early murine development. When extended to other ages and brain regions, we found this core molecular signature was conserved, but most cellular perturbations were largely region and age specific. Our results provide new insights into phenotypic variability, shared versus background- or allele specific effects associated with the impact of *Chd8* haploinsufficiency on mouse forebrain development.

Disclosures: N. Seban: None. **C.P. Canales:** None. **M. Tabbaa:** None. **S.A. Lozano:** None. **K. Cichewicz:** None. **E. Fenton:** None. **N. Chu:** None. **S. Jung:** None. **D. Rahbarian:** None. **A. Kopley-Smith:** None. **D. Fioravante:** None. **A.S. Nord:** None.

Poster

PSTR370: Animal Models of Autism

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.27/A55

Topic: A.06. Developmental Disorders

Support: R01: R01NS105000
Robert Wood Johnson Foundation grant #74260

Title: Expression Level Impact of Developmental Cc2d1a Reduction as a Sex-Specific Mouse Model of ASD/ID and growth deficits

Authors: *A. T. HELLER[1], A. BHATTACHARYA[2], E. SUZUKI[3], P. ROUTRAY[3], M. MANZINI[4];

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Abstract: Intellectual disability (ID) and Autism Spectrum Disorder (ASD) exhibit notable male diagnostic bias (4:1 for ASD, 2:1 for ID) with complex genetic heterogeneity. Previous research identified *CC2D1A* loss of function (LOF) as a cause of 100% penetrant ID, and highly penetrant ASD. While existing *Cc2d1a* conditional knockout (cKO) mouse models avoid early postnatal lethality of global knockouts, their conditional limitations inadequately represent patients with developmental *CC2D1A LOF*. In generating mice where CC2D1A is tagged with V5-HA epitope tags we found that this modification results in protein degradation and a hypomorph allele. Homozygous V5-HA mice ($Cc2d1a^{VH/VH}$) survive despite ~86% CC2D1A reduction but do not show cKO ASD/ID-like behaviors. To explore phenotypic penetrance thresholds, we crossed this line with a null allele, creating a more severe hypomorph ($Cc2d1a^{-/VH}$) with ~90% protein reduction. These mice are viable and fertile without major sensory-motor deficits. $Cc2d1a^{-/VH}$ females show developmental stunting at the onset of puberty but normal behavior. While $Cc2d1a^{-/VH}$ males exhibit deficits in sociability, social novelty preference, and spatial memory acquisition as we had found in forebrain-specific cKOs. $Cc2d1a^{-/VH}$ males also show sex-specific cortical CaMKII activation deficits and lower NPY/AgRP mRNA expression in the arcuate nucleus which also suggests hypothalamic signaling dysregulation in mutant males. This severe developmental hypomorph model reveals unique neuronal mechanisms driving sex-specific ASD/ID-like phenotypes within *Cc2d1a* reduction, offering novel insights into what factors may be at play within the ASD and ID diagnostic sex bias.

Disclosures: **A.T. Heller:** A. Employment/Salary (full or part-time): Rutgers University. **A. Bhattacharya:** None. **E. Suzuki:** None. **P. Routray:** None. **M. Manzini:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.01/A56

Topic: A.06. Developmental Disorders

Support: JPB Foundation Postdoctoral Fellowship (G.F.)
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R01MH133066
Simons Foundation Autism Research Initiative
Japan Society for the Promotion of Science (JSPS) Overseas Research Fellowships (Y.O.)

Title: The role of altered noradrenergic signaling in motor dysfunction in Rett Syndrome

Authors: *G. FERNANDES[1], H. SUGIHARA[1], Y. OSAKO[1], A. NOVIELLI[1], M. SUR[2];
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Abstract: The acquisition and execution of motor skills are essential for everyday life. Disruption of these motor skills is one of the most debilitating symptoms of Rett Syndrome, leading to the loss of purposeful hand use and the development of repetitive movements, rigidity, and dystonia. The motor cortex, which is crucial for voluntary movement and motor learning, is regulated by norepinephrine released from the Locus Coeruleus (LC). Loss of MeCP2, a gene linked to Rett Syndrome, reduces global norepinephrine release. However, the effects of MeCP2 loss on phasic LC activity and its role in motor learning remain unexplored. To investigate this, we combined *in vivo* 2-photon imaging with a motor learning task, where mice were trained to execute a motor response to a tone in order to receive a water reward. MeCP2 loss in the LC reduced axonal activity and disrupted norepinephrine release during both the motor execution and post-reinforcement phases. Consequently, mice with LC-specific MeCP2 loss exhibited delayed motor learning, increased movement jerkiness, and failed to develop consistent, goal-directed movements. This indicates a crucial role for LC-MeCP2 in both the accuracy of behavioral performance and the execution of goal-driven motor movements. Further, these shifts in the spatiotemporal dynamics of norepinephrine signaling modulated the ensemble activity and synchrony of motor cortex neurons, thus altering the cortical representation of motor learning. Together, these findings uncover a novel role for the norepinephrine system in the development of motor control and identify a potential therapeutic target for mitigating motor dysfunction in Rett Syndrome.

Disclosures: G. Fernandes: None. **H. Sugihara:** None. **Y. Osako:** None. **A. Novielli:** None. **M. Sur:** None.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.02/A57

Topic: A.06. Developmental Disorders

Support: AMED 23gm1310008 J
JSPS KAKENH JP22K15201
JSPS KAKENHI JP23H04170

Title: Possible involvement of endogenous DNA-induced microglial activation in the pathogenesis of Rett syndrome

Authors: *H. NAKASHIMA[1], K. NAKASHIMA[2];
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Abstract: Rett syndrome (RTT) is a severe progressive neurodevelopmental disorder in females, mainly caused by mutations in the X-linked gene encoding *methyl-CpG binding protein 2* (*MeCP2*). Substantial evidence has shown that morphological and functional abnormalities of neurons are involved in RTT pathogenesis, therefore, it has been considered that symptoms of RTT are attributed to neuron-intrinsic dysfunction in the patients. However, increasing evidence has suggested that glial abnormalities associate with the pathogenesis of RTT, e.g., activation of microglia, brain-resident immune cells resembling macrophage, has been observed in MeCP2 knock-out (KO) RTT model mice, whereas the underlying mechanisms still remains unknown. We have previously shown that the expression of retrotransposon LINE1 (L1) and the transposition of its reverse transcript (cDNA) into the genome are increased in MeCP2-KO mouse brains. However, it has recently been shown that L1-cDNA activate innate immune responses via cytosolic DNA sensors. Based on these findings, we hypothesized that accumulation of L1-cDNA leads to aberrant microglial activation in MeCP2-deficient brains through DNA sensor pathways. To test this, we treated MeCP2-KO mice with a reverse transcriptase inhibitor (RTi) to suppress L1-cDNA production. RTi treatment significantly reduced microglial activation in MeCP2-KO brains. Furthermore, MeCP2-deficient mice lacking DNA sensors exhibited amelioration of RTT-like phenotypes, supporting the involvement of this signaling axis. These results suggest that the L1-cDNA-DNA sensor-microglia activation pathway contributes to RTT pathogenesis and may represent a novel therapeutic target for RTT.

Disclosures: H. Nakashima: None. **K. Nakashima:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.03/A58

Topic: A.06. Developmental Disorders

Title: Elpis study: plasma biomarkers in CDKL5 deficiency disorder with focus on α-tubulin PTMs

Authors: *V. PERGHER, C. MALTBY, E. PASCHEN, M. BIANCHI;
Ulysses Neurosci. Ltd, Dublin, Ireland

Abstract: CDKL5 Deficiency Disorder (CDD) is a rare X-linked Developmental and epileptic encephalopathy (DEE) characterised by early-onset epilepsy and severe cognitive and motor impairments. The disease is due to heterogeneous mutations of the Cdkl5 gene resulting in lack of function of the CDKL5 protein. CDKL5, a serine-threonine kinase, is widely recognized as a key modulator of microtubule dynamics which has been therefore involved in the pathogenesis and treatment of CDD. Microtubules, composed of α− and β−tubulin dimers, play a key role in synaptic plasticity, critical during early development and maturation of the central nervous system.
ELPIS is a global clinical fluid biomarker study aimed to investigate alpha-tubulin post-

translational modifications (PTMs) as potential plasma biomarkers of CDD pathology. Alpha-tubulin PTMs such as Acetylated alpha-tubulin and Tyrosinated/detyrosinated alpha-tubulin are associated with less or more dynamic microtubules, respectively.ELPIS international reach is unique in rare DDEs and the study currently has sites in Ireland, Italy, Portugal, Spain and Brazil with ongoing expansions to Germany, South-America, Israel and Australia. Once the availability of a clinical centre, two paediatric nurses, and a site coordinator is confirmed, the workflow starts with the shipment of kit containing the vials for the human plasma collection with standardized protocols. Plasma is collected in barcoded samples and shipped back to our laboratories for biobanking and analysis. Molecular analyses is conducted in plasma from CDD patients compared to neurotypical controls, to measure levels of acetylated α-tubulin normalised to total α-tubulin (Acet/Tot-Tub) and tyrosinated α-tubulin normalised to de-tyrosinated α-tubulin (Tyr/Glu-Tub) via infrared Western Blot (IRWB). Neurofilament light chain (Nf-L), Brain Derived Neurotrophic Factor (BDNF), and a panel of inflammatory cytokines and chemokines was also assessed via electrochemiluminescence ELISA (Mesoscale Diagnostics). Our data show significant overexpression of Acet-Tub/Tot-Tub in the plasma of CDD patients compared to controls suggesting decreased microtubule dynamics. Nf-L and BDNF showed trend towards increase and decrease, respectively, with important variability. Interleukin-10 is significantly increased indicating immune dysregulation. Acet-Tub/Tot shows high potential as a biomarker of disease progression in CCD linked to the pathogenesis of the disease.

Disclosures: V. Pergher: A. Employment/Salary (full or part-time): Ulysses Neuroscience Limited. **C. Maltby:** A. Employment/Salary (full or part-time): Ulysses Neuroscience Limited. **E. Paschen:** A. Employment/Salary (full or part-time): Ulysses Neuroscience Limited. **M. Bianchi:** A. Employment/Salary (full or part-time): Ulysses Neuroscience Limited.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.04/A59

Topic: A.06. Developmental Disorders

Support: NIH NINDS 1R01NS087142/01

Title: Molecular consequences of altering MeCP2 levels driving neurological deficits

Authors: *A. ENGSTROM[1], M. DIAS[1], Y. LI[2], A. ANDERSON[3], H. YALAMANCHILI[1], H. Y. ZOGHBI[4];
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Abstract: Methyl CpG binding protein (MeCP2), binds broadly across the genome to regulated proper transcription, and critical for neuronal function. The levels of MeCP2 are tightly regulated

during mammalian brain development and in the mature brain. The severity of neurological symptoms observed in patients correlates with the degree of change, both increased and decreased. Loss-of-function mutations result in Rett Syndrome (RTT) while mutations that retain some amount of MeCP2 protein are milder. The duplication of *MECP2* leads to *MECP2* Duplication Syndrome (MDS), a neurodevelopmental disorder similar to RTT. Interestingly, patients with even higher levels of MeCP2*,* like those with triplications of the gene, present with even more severe symptoms. This suggests that the mechanism of MeCP2 function in the brain is acutely influenced by dosage. However, the molecular consequences of varying MeCP2 levels *in vivo* are poorly understood. To address this, we utilize an allelic series of mouse models, including one mouse line that lacks MeCP2 completely and four mouse lines that express a gradient of MeCP2 ranging from 50-350% of wildtype protein. To identify how these changes in levels impact MeCP2 DNA-binding, the chromatin landscape, and resultant gene expression changes, we performed both CUT&RUN and RNAseq on the frontal cortex of mice across the allelic series prior to symptom onset. Our data suggest that increasing MeCP2 levels results in increased MeCP2 binding globally and at known MeCP2 binding sites. We determined that the increase of MeCP2 binding is not specific to a certain functional genomic region (introns, coding regions, promoters, major satellite, etc.) and that at all dosages, MeCP2 has a slight preference for promoter regions. Our transcriptional analysis argues that, as MeCP2 levels move further from wildtype, differentially expressed genes become exacerbated, as well as an additive effect where new genes are being dysregulated. We identify a core set of "dosage dependent genes" that are dysregulated across all MeCP2 dosage models in a linear and progressive manner. Additionally, we found that the majority of differently expressed genes showed differential binding of MeCP2 at the promoter. Through further integrative analysis of the RNAseq, MeCP2 binding, and the changing chromatin landscape across the allelic series, we will determine the relationship between the MeCP2 binding alterations and transcription to elucidate the dosage dependence of MeCP2 and advance our understanding of its molecular function.

Disclosures: A. Engstrom: None. **M. Dias:** None. **Y. Li:** None. **A. Anderson:** None. **H. Yalamanchili:** None. **H.Y. Zoghbi:** None.

Poster

PSTR371: Developmental Disorders

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Topic: A.06. Developmental Disorders

Support: NINDS R01 NS057819

Title: Functional genomic screens identify CSNK2A1 as novel drug target for modulating MeCP2

Authors: *A. ANDERSON[1], S. VEERARAGAVAN[2], H. Y. ZOGHBI[3];
[1]Baylor Col. of Med., Houston, TX; [2]Baylor Col. of medicine, Houston, TX; [3]Mol. and Human Genet., Baylor Col. of Medicine/HHMI, Houston, TX

Abstract: The brain is extremely sensitive to levels of methyl-CpG binding protein 2 (MeCP2), a critical protein for neuronal function. Loss-of-function mutations in *MECP2* can reduce MeCP2 levels and lead to Rett syndrome (RTT), whereas duplication of the *MECP2* locus increases MeCP2 levels and causes MECP2 duplication syndrome (MDS). Both RTT and MDS are severe neurological disorders. Normalizing MeCP2 levels in the brain—either by increasing MeCP2 in RTT models or decreasing it in MDS models—can rescue key symptoms of both disorders. Therefore, understanding the molecular pathways regulating MeCP2 stability is essential for therapeutic development.To investigate MeCP2 regulation, we developed tools to perform two genome-wide CRISPR knockout (KO) screens to identify proteins that either increase or decrease MeCP2 levels. These screens identified approximately 2,000 potential MeCP2 regulators. We validated top hits by assessing their effects on endogenous MeCP2 protein levels using independent gRNAs and small-molecule inhibitors. Casein kinase 2 alpha subunit 1 (*CSNK2A1*) emerged as a top hit in both screens as a stabilizer of MeCP2, where KO of *CSNK2A1* reduced MeCP2 protein levels.Interestingly, mutations in *CSNK2A1* are associated with Okur-Chung neurodevelopmental syndrome (OCNDS), which shares clinical similarities with RTT. Here, we show that CSNK2A1 physically interacts with MeCP2 in mouse brain tissue and human iPSC-derived glutamatergic neurons and phosphorylates MeCP2 on distinct serine residues. By genetically reducing *Csnk2a1* in a mouse model of MDS, we are able to lower MeCP2 levels in specific brain regions and rescue impairments in activity, motor coordination, and stereotypies. Future studies will investigate whether pharmacological inhibition of *Csnk2a1* in vivo produces similar effects. The findings from our genetic screens reveal promising drug targets for treating MeCP2-related disorders and potential strategies for treating other neurodevelopmental syndromes.

Disclosures: A. Anderson: None. **S. veeraragavan:** None. **H.Y. Zoghbi:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

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Program #/Poster #: PSTR371.06/A61

Topic: A.06. Developmental Disorders

Support: IRSF grant 3807
Marguerite-Marie Delacroix Foundation PhD Fellowship
Generet Award for Rare Disease Research
FWO Flanders Clinical Investigator

Title: In vivo xenotransplantation of patient iPSC-derived neurons in MECP2 neurodevelopmental disorders

Authors: *N. MERCKX[1], E. ERKOL[1], L. BOUBAKAR[1], R. IWATA[1], H. VAN ESCH[2,3], P. VANDERHAEGHEN[1];
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Abstract: Brain development is controlled by epigenetic programs that specify the transcription of genes needed for development, yet the mechanisms underlying this regulation remain incompletely understood. These programs involve both gene silencing and activation, mediated in part by DNA methylation. **Methyl-CpG-binding protein 2 (MECP2)** is a crucial epigenetic regulator that binds methylated DNA and influences transcription through diverse, context-dependent mechanisms. MECP2 is essential for proper brain development and neuronal function. Alterations in MECP2 underlie severe neurodevelopmental disorders (NDDs) such as **Rett syndrome (RTT) and MECP2 duplication syndrome (MDS)**, highlighting the critical importance of this X-linked gene in brain development. Ideally, studying RTT and MDS would involve direct analysis of patient brain tissue. However, such samples are scarce and limited to postmortem material, reflecting only late symptomatic stages. This limits investigation of early pathogenic mechanisms during development, when critical disruptions occur.
While animal models have provided important insights into MECP2 function, translating these findings to patients is limited due to **species-specific differences**. Human cortical neurons are more complex and mature more slowly than mouse neurons, emphasizing the need for human-specific models of early brain development, particularly in disorders like RTT and MDS that affect neuronal maturation.
To address this need, we established an **in vivo xenotransplantation model** by transplanting patient iPSC-derived cortical neurons into neonatal mouse cortex for MDS and RTT. This enables the study of human neurons in a physiologically relevant brain environment and integration into mouse circuits, while preserving human-specific timing of maturation. Importantly, this approach provides access to early disease stages, capturing the onset of molecular and structural changes as symptoms emerge and progress.
Using isogenic control, duplication, and knockout lines, we combined single-nucleus RNA sequencing (snRNA-seq) and high-resolution microscopy to investigate MECP2 dosage effects at defined timepoints post-transplantation. We observed global transcriptional dysregulation in MECP2-KO and MDS neurons, affecting genes involved in neuronal function and RNA splicing. In parallel, we detected altered dendritic spine maturation in both loss- and gain-of-function models.
This **human in vivo neuronal model** provides a platform to study early stages of MECP2-related disease and supports therapeutic target discovery relevant to a broader range of NDDs.

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Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.07/A62

Topic: A.06. Developmental Disorders

Support: NIH-U54HD104461
NIH-F31HD113236-01

Title: A cell type specific switch in GluN2C/D containing NMDA receptors drives cortical circuit hyperexcitability in a Fragile X Syndrome mouse model

Authors: *A. SHEDD, J. R. GIBSON, K. M. HUBER;
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Abstract: Fragile X Syndrome (FXS) is a leading monogenic cause of Autism Spectrum Disorder (ASD) and inherited intellectual disability. Patients experience cognitive and social impairments, altered sensory processing and sensitivity, and epilepsy. EEG studies in patients with FXS and the mouse model of FXS, the *Fmr1* knockout (KO), reveal hyperexcitability and deficits in sensory-driven cortical circuit synchrony. In acute slices of somatosensory cortex from *Fmr1* KO mice, hyperexcitability and altered circuit timing can be measured by prolonged persistent activity states, called UP states. The UP state assay provides a reduced preparation to study mechanisms of local cortical circuit dysfunction in *Fmr1* KO mice and results may provide insight in circuit mechanisms of the altered EEG in FXS. Using the UP state assay, I discovered that GluN2C/D subunit containing NMDA receptors (NMDARs) contribute to hyperexcitability in the *Fmr1* KO. Specifically, a GluN2C/D positive allosteric modulator (CIQ) increases UP state duration, while a negative allosteric modulator (NAB-14) rescues UP state duration. Importantly, these interventions only affect UP states in the *Fmr1* KO and not wildtype (WT) littermates. Additionally, pretreatment with a GluN2C/D antagonist reduces sensory hypersensitivity in the *Fmr1* KO, as measured by audiogenic seizures. In the cortex, GluN2C or D containing NMDARs are expressed in either astrocytes or inhibitory neurons, respectively. To determine if GluN2C/D containing NMDARs are dysfunctional in *Fmr1* KO neurons, I measured the sensitivity of evoked, NMDAR-mediated excitatory synaptic currents (EPSCs) to NAB-14 on excitatory pyramidal (PYR) neurons or parvalbumin-positive (PV) inhibitory neurons in Layer 5 of somatosensory cortex. In WT mice, NAB-14 had reduced EPSC amplitude in PV neurons and had no effect on EPSCs in PYR neurons, as expected. However, in *Fmr1* KO mice, NAB-14 reduced NMDAR-EPSCs in PYR neurons and had no effect on PV neurons. These results suggest a functional misexpression of GluN2C/D NMDARs in *Fmr1* KO L5 PYR neurons. In support of this conclusion, CIQ enhances intrinsic excitability of *Fmr1* KO, but not WT, PYR neurons. Taken together, my data reveals a cell type specific switch in functional GluN2C/D NMDARs in *Fmr1* KO cortex that drives hyperexcitable circuits. My ongoing studies aim to examine cell-type specific expression of GluN2C/D-NMDAR RNA (*grin2c/d*) and determine the contribution of GluN2C/D NMDARs to sensory processing phenotypes measured with EEG. These results provide insight into the cellular basis of GluN2C/D NMDAR dysfunction in FXS and suggest that GluN2C/D may be a therapeutic target for FXS.

Disclosures: A. Shedd: None. **J.R. Gibson:** None. **K.M. Huber:** None.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.08/B1

Topic: A.06. Developmental Disorders

Support: Wellcome Trust 218461/Z/19/Z

Title: Whole-neuron mapping of synaptic architecture in Fragile X Syndrome

Authors: ***V. MENNE**[1,2], F. RICCIO[2], A. MASET[2], G. NEVES[2], I. LIEBERAM[2], J. BURRONE[2];
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Abstract: Excitation-inhibition (E/I) balance is critical for maintaining healthy neuronal network function and is frequently disrupted in neurodevelopmental disorders such as Fragile X Syndrome (FXS). Although functional alterations in E/I balance have been widely studied in FXS, particularly through electrophysiological methods, the subcellular architecture of excitatory and inhibitory synapses remains poorly understood. This study addresses this gap by investigating the spatial distribution of excitatory (PSD95) and inhibitory (Gephyrin) postsynaptic sites at single-synapse resolution.
To maximise translatability, we employ both the Fmr1 KO mouse and patient iPSC-derived excitatory and inhibitory neurons. Human NGN2-induced cortical-like glutamatergic neurons and ASCL1/DLX2-induced somatostatin-expressing GABAergic interneurons are co-cultured to form functional networks. We developed a system for sparse labelling in both mouse brain and human co-culture. Using genetic engineering via in utero AAV delivery in mice and a combination of AAVs and transposase-based integration in human cultures, we express intrabodies targeting PSD95 and Gephyrin. Mice are sacrificed at postnatal days 7, 14, and 21; human co-cultures fixed after 6 weeks in vitro. Sparse labelling enabled whole-neuron imaging via confocal microscopy and analysis of subcellular compartments including dendrites, soma, and axon initial segment (AIS). Synaptic distribution and quantification were performed using Imaris software. Mapping of synapses across compartments is ongoing in both systems. Preliminary results in mice show reduced inhibitory synapse density at the AIS in KO mice, relative to controls. In agreement with prior work, we observed AIS elongation, which may partly explain this reduction. Notably, AIS elongation was not observed in human neurons.
We developed a platform for sparse labelling of excitatory and inhibitory synapses in both in vivo mouse brain and in vitro human networks, enabling single-neuron resolution imaging of synaptic architecture. Preliminary application to FXS reveals altered inhibitory synapse organization in mouse neurons and suggests species-specific differences compared to human models. This approach provides a powerful framework for uncovering fundamental principles of synaptic organization and how they are disrupted in neurodevelopmental disorders.

Disclosures: V. Menne: None. **F. Riccio:** None. **A. Maset:** None. **G. Neves:** None. **I. Lieberam:** None. **J. Burrone:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.09/B2

Topic: A.06. Developmental Disorders

Support: R01NS117597 (NIH-NINDS),
R01HD054453 (NIH-NICHD)
Department of Defense (DOD, 13196175)
the Center for Autism Research and Treatment at UCLA
R01HD108370
P50HD103557-05
R01MH121521

Title: Translatome profiling reveals opposing alterations in inhibitory and excitatory neurons of Fragile X mice and identifies EPAC2 as a therapeutic target

Authors: *A. SURESH[1], T. NOMURA[2], N. KOURDOUGLI[4], J. E. BUTH[5], S. MIRANDA[6], R. ARAYA[7], M. J. GANDAL[8], A. CONTRACTOR[3], C. PORTERA-CAILLIAU[4];
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Abstract: Fragile X Syndrome (FXS) is a prototypical neurodevelopmental condition characterized by intellectual disability, autistic traits, and atypical sensory processing. It is caused by transcriptional silencing of the FMR1 gene, which leads to the near complete loss of fragile X messenger ribonucleoprotein (FMRP), an RNA binding protein that functions as a translational suppressor of several hundred proteins. Exactly how loss of FMRP and the resulting dysregulation of molecular signaling pathways affects brain circuit function remains an area of active investigation. To date, it has been particularly difficult to link FMRP with molecular signaling pathways that regulate synaptic strength and dendritic spine turnover, both of which are affected in Fmr1 knockout (KO) mice, the principal animal model of FXS. In parallel, other efforts have implicated changes in excitatory and inhibitory circuits in the etiology of FXS, including reduced firing and density of parvalbumin (PV) neurons, the major subtype of inhibitory interneurons in the cerebral cortex. To investigate whether loss of FMRP similarly affects the transcriptome of excitatory and inhibitory neurons, we used a Ribo-Tag approach to isolate mRNA specifically from Ca2+/calmodulin-dependent protein kinase II alpha (CAMK2a) and PV neurons in primary somatosensory (S1) and visual (V1) cortices of adult Fmr1 KO mice and wild-type (WT) controls. This revealed hundreds of differentially expressed (DE) between Fmr1 KO and WT mice, especially in excitatory neurons, but without clear region-specific differences. Intersectional analysis identified 194 DE genes that were shared between both CAMK2a and PV neurons, including several autism risk genes and genes known to be regulated by FMRP. Among these shared genes, we identified Epac2 (a.k.a., Rapgef4), a cAMP dependent

guanine-exchange factor, as the only DE gene (upregulated in Fmr1 KO mice) that is also an autism risk gene, a target of FMRP, and enriched in brain tissue, making it an ideal target for therapeutic intervention. Overactivation of EPAC2 is known to to induce spine shrinkage and turnover, as well as lower GLUA2 AMPA receptor content. Strikingly, our group and others have reported similar synaptic phenotypes in Fmr1 KO mice. We therefore used a combination of pharmacologic and genetic approaches to inhibit EPAC2 in Fmr1 KO mice and evaluate behavioral, systems, and physiological synaptic phenotypes and identify EPAC2 as a potential therapeutic target for FXS.

Disclosures: A. Suresh: None. **T. Nomura:** None. **N. Kourdougli:** None. **J.E. Buth:** None. **S. Miranda:** None. **R. Araya:** None. **M.J. Gandal:** None. **A. Contractor:** None. **C. Portera-Cailliau:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.10/B3

Topic: A.06. Developmental Disorders

Support: Swank Foundation, R21NS117112 (RS)

Title: Medial Septum Diagonal Band of Broca Stimulation Rescues contextual fear Deficit Observed in FMR1-KO Mice

Authors: *M. OUARDOUZ[1], A. E. HERNAN[2], M. MAHONEY[3], R. SCOTT[4];
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Abstract: Mutations that lead to loss of functional Fragile X Ribonucleoprotein 1 protein (FMR1P) are the main cause of inherited mental retardation in Fragile X Syndrome (FXS). Abnormal dendritic spines in FXS patients and FMR1 KO mice lead to altered synaptic function, plasticity, and neuronal excitability, contributing to cognitive and behavioral issues. Our previous experiments on FMR1-KO mice showed that medial septum/diagonal band of broca (MSDBB) stimulation improved recognition memory, sociability, spatial learning, fear memory, and long-term plasticity (LTP) in the hippocampal CA1 region. The objective of this study is to test the effect of MSDBB stimulation on hippocampal plasticity and contextual fear learning. Wild-type mice and FMR1-KO mice were used. For deep brain stimulation, a bipolar stimulating electrode was implanted in the MSDB under isoflurane anesthesia. Three groups Control, Fmr1-KO and Fmr1 KO stim (30 minutes stimulation of MSDBB at 35 Hz before behavioral test) were subjected contextual fear conditioning. All behavioral trials were video recorded and analyzed offline. The percent of freezing time was measured using ezTrack software. We also explored the effect of MSDBB stimulation on excitatory synaptic transmission and short-term plasticity in

CA1 hippocampus using slice preparation from different animals. MSDBB stimulation at 35 Hz for 30 minutes was able to rescue deficit of contextual fear observed in Fmr1 KO mice. Experiment on synaptic transmission and short-term plasticity showed an impairment in the synaptic input/output relationship and short-term plasticity that is rescued by MSDBB stimulation. Our overall findings provide strong proof of concept that the MSDB represents an excellent target for brain stimulation to rescue cognitive and plasticity deficits in FXS.

Disclosures: M. Ouardouz: None. **A.E. Hernan:** None. **M. Mahoney:** None. **R. Scott:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.11/B4

Topic: A.06. Developmental Disorders

Support: R01 HD055453 (NIH-NICHD)
R01 HD108370 (NIH, NICHD)
R01 NS117597 (NIH- NINDS)

Title: Differences in receptive field size and size tuning in primary visual cortex of Fragile X mice

Authors: *I. A. TELLO-GARCIA[1], C. PORTERA-CAILLIAU[1,2];
[1]Dept. of Neurol., [2]Dept. of Neurobio., UCLA, Los Angeles, CA

Abstract: Atypical sensory processing is a hallmark of Fragile X syndrome (FXS). Sensory hypersensitivity, and how it contributes to gaze aversion, anxiety, attention deficit, and learning delay, has been studied extensively. In contrast, differences in visual integration in FXS, which are also prominent in FXS/autism, are less well understood. We investigated differences in circuit function as it relates to contextual modulation in the primary visual cortex (V1) of *Fmr1* knockout (KO) mice, a well-established model of FXS. We first used *in vivo* two-photon calcium imaging in head-fixed adult mice to record from pyramidal (Pyr) neurons in layer 2/3 of V1 and tested different mechanisms of visual integration like receptive field (RF) size, size and orientation tuning, and surround suppression. Strikingly, aside from previously reported reduced visual-evoked responses in V1, the main phenotype in *Fmr1* KO mice was related to RF size and size tuning, not surround suppression. Pyr neurons in V1 of *Fmr1* KO mice had significantly larger receptive fields than those in WT mice ($69.3^{o} \pm 10.3$ vs. $44.5^{o} \pm 8.5$), and larger stimulus sizes evoked stronger responses (Z-score) in *Fmr1* KO mice than in WT controls (peak at 40° vs. 20°). To investigate the contributions of inhibitory interneuron populations within the V1 microcircuit, we also recorded from parvalbumin (PV) and somatostatin (SST) cells in PV-Cre and SST-Cre mice. Preliminary data reveal hypoactivity in PV interneurons and reduced orientation selectivity in *Fmr1* KO mice, consistent with prior findings. Experiments examining SST interneuron function are ongoing. Collectively, these findings will guide an integrative

circuit-level approach to elucidate the role of local inhibitory circuits on visual integration deficits in *Fmr1* KO mice. Future studies will address whether these changes in size tuning translate into behavioral deficits for *Fmr1* KO mice.

Disclosures: I.A. Tello-Garcia: None. **C. Portera-Cailliau:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.12/Web Only

Topic: A.06. Developmental Disorders

Support: USDA 2018-67001-28266
DOD HT9425-23-1-0076

Title: Soy-based purified ingredient diet affects mouse gut permeability and the microbiome in fragile X mice

Authors: *C. J. WESTMARK;
Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Gastrointestinal problems including vomiting, reflux, flatulence, diarrhea, constipation and colic are common comorbidities in fragile X syndrome. There is accumulating evidence suggesting that leaky gut syndrome causes neurological phenotypes. Although fragile X messenger ribonucleoprotein is ubiquitously expressed, there is a dearth of knowledge regarding its role outside of the brain including effects on gut dysfunction in fragile X. The aim of this study was to generate novel data on gastrointestinal barrier function and the gut microbiome in response to *Fmr1* genotype, sex and diet in mice.**Methods:** $Fmr1^{KO}$ male mice and littermate controls in an FVB background were maintained on two purified ingredient diets (AIN-93G with casein protein versus soy protein isolate) versus two standard chows (Teklad 2019 with wheat, corn and yeast protein versus Purina 5015 with wheat, soy, corn, yeast and whey protein sources). Gut permeability was quantified by FITC-dextran levels in blood plasma. The cecal microbiome was identified by 16S rRNA sequencing. In addition, gut permeability was tested in $Fmr1^{KO}$ mice in the C57BL/6 J background maintained on casein- and soy protein isolate-based AIN-93G versus Teklad 2019.**Results:** Knockout of the *Fmr1* gene in FVB mice did not affect gut permeability. Soy protein isolate-based AIN-93G increased gut permeability. Beta-diversity of the cecal microbiome was significantly altered as a function of the four test diets. *Akkermansia_muciniphila* was increased in $Fmr1^{KO}$ mice fed AIN-93G while unnamed species within the genus *Anaerovorax* and family Ruminococcaceae were increased and the order Clostridales decreased in $Fmr1^{KO}$ mice fed AIN-93G/soy. $Fmr1^{KO}$ mice in the C57BL/6 J background exhibited increased gut permeability in response to soy protein.**Discussion:** These findings regarding the effects of diet on gut permeability and the microbiome have important implications for experimental design. Single-source diets are

ubiquitously used to maintain laboratory animals for medical research and feed details are frequently not reported in publications. Diet/phenotype interactions could have a large impact on inter-laboratory replicability in premedical research. For infants with fragile X, early-life diet could impact the severity of disease outcomes.

Disclosures: C.J. Westmark: None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.13/B5

Topic: A.06. Developmental Disorders

Support: NIH Grant R15S088776.

Title: Early-life stress and fmr1 deletion alter synaptic and behavioral trajectories in a mouse model of fragile x syndrome

Authors: *K. J. BLANDIN[1], C. LAU[2], T. BRADISH[1], J. J. THAYIL[1], C. ENYERIBE[3], M. J. HEMMERSMEIER[1], K. J. LALLY[1], J. N. LUGO, Jr.[4];
[1]Baylor Univ., Waco, TX; [2]Baylor Univ., Shoreacres, TX; [3]Baylor Univ., Frisco, TX; [4]Psychology and Neurosci., Baylor Univ., Mc Gregor, TX

Abstract: Fragile X syndrome (FXS), the most heritable cause of intellectual disability and autism spectrum disorder (ASD), affects 1 in 7,000 males and 1 in 11,000 females. FXS results from reduced translation of the Fragile X messenger ribonucleoprotein 1 (*FMR1*) gene and loss of Fragile X Messenger Ribonucleoprotein (FMRP), which leads to immature dendritic spines. While genetics plays a central role, environmental factors like early-life stress (ES) can exacerbate cognitive and behavioral deficits. Although stress in *Fmr1* knockout (KO) mice indicates heightened prenatal sensitivity, early postnatal effects still require further investigation. Both *Fmr1* KO mice and stress-exposed models demonstrate behavioral deficits linked to disrupted neuronal connectivity and impaired synaptic plasticity. This study examined the effects of ES on *Fmr1* KO and wildtype (WT) C57BL/6J male mice, utilizing a limited bedding and nesting paradigm from postnatal days 2 to 9. Corticosterone levels were assessed acutely and over the long term. In adulthood, synaptic spine density within the primary somatosensory motor cortex was evaluated via Golgi-Cox staining, and mice underwent behavioral testing to determine lasting deficits. Corticosterone was unaffected acutely and into adulthood. *Fmr1* KO mice exhibited heightened locomotor activity, increased repetitive behavior and decreased anxiety-like behaviors, learning deficits, and altered communication. Stress exposure resulted in learning impairments, decreased anxiety-like behavior, increased activity, heightened startle response, increased repetitive behaviors, and changes in ultrasonic vocalization call type. Early-life stress superimposed on the KO condition enhanced locomotor activity. Both stress and *Fmr1* deletion elevated synaptic spine density, though stress in KO mice showed no significant

increase, suggesting a potential ceiling effect in KO mice. These results indicate that early-life stress has a harmful impact on its own but does not exacerbate ASD-like behaviors in the absence of *Fmr1*. The corticosterone findings indicate a potential blunted hormone response from habituation across genotypes. These findings highlight the long-term effects of early-life stress, Fmr1 deletion, and their combined impact on brain development.

Disclosures: K.J. Blandin: None. **C. Lau:** None. **T. Bradish:** None. **J.J. Thayil:** None. **C. Enyeribe:** None. **M.J. Hemmersmeier:** None. **K.J. Lally:** None. **J.N. Lugo:** None.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.14/B6

Topic: A.06. Developmental Disorders

Support: NIH U01 NS096767

Title: Developmental trajectories for neural oscillatory activity in young children with fragile x syndrome.

Authors: *E. LEE[1], E. BERRY-KRAVIS[3], E. AUGER[4], B. ARLEDGE[4], L. ETHRIDGE[2]; [1]Psychology, [2]Dept. of Psychology, Univ. of Oklahoma, Norman, OK; [3]Dept. of Pediatrics, Neurolog. Sciences, and Biochem., Rush Univ. Med. Ctr., Chicago, IL; [4]Dept. of Psychology, The Univ. of Oklahoma, Norman, OK

Abstract: Fragile X Syndrome (FXS) is a monogenic neurodevelopmental disorder associated with intellectual disability and a range of cognitive, behavioral, and language challenges. This study leverages longitudinal EEG data from the FXLEARN multisite clinical trial to examine how neural oscillatory activity develops in FXS in early childhood, a critical period for cognitive and language development. While prior research in adolescents and adults with FXS has identified elevated theta and reduced alpha power, little is known about the developmental trajectories of these features in younger children.Three minutes of eyes-open resting EEG recording were collected at multiple timepoints over a 10-month period from 18 children with FXS aged 3-8 years, all of whom had FMR1 full mutation and completed at least three of four study visits. Whole-head relative power in the theta (4-8 HZ) and alpha (8-12Hz) frequency bands, which are commonly linked to attention, memory, and learning, was analyzed using a mixed-effects regression model that accounted for both within-subject variability and varying baseline ages.Preliminary results showed that theta power significantly decreased with baseline age, $t(23.95)=-2.31$, $p<.05$, consistent with typical developmental patterns. However, alpha power remained relatively flat across time and baseline age, diverging from the expected increase seen in typically developing children. This lack of alpha growth may indicate a delayed maturation of alpha oscillatory activity. Results may represent a potential biomarker for identifying critical periods of intervention based on cognitive development. Planned additional

analyses include using the Fitting Oscillations and One Over F (FOOOF) algorithm to isolate periodic and aperiodic contributions to theta/alpha ratio and shifts in developmental timing as well as their relationship to cognitive and language outcomes.By capturing early changes in neural activity and their relation to early cognition, this study lays essential groundwork for future research on EEG based biomarkers and informs ongoing efforts to tailor early interventions for children with FXS.

Disclosures: E. Lee: None. **E. Berry-Kravis:** None. **E. Auger:** None. **B. Arledge:** None. **L. Ethridge:** None.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.15/B7

Topic: A.06. Developmental Disorders

Support: Jim and Betty Ann Rodgers Chair Fund
FSU Council on Creativity and Research

Title: Dysregulation of Kappa Opioid Receptor Signaling Contributes to Motivational Deficits in a Mouse Model of Fragile X Syndrome

Authors: M. X. TRUPIANO[1], A. A. NEMEC[1], C. VIED[2], P. G. BHIDE[1,4], *D. M. MC CARTHY[3,4];
[1]Dept. of Biomed. Sci., [3]Biomed. Sci., [2]Florida State Univ. Col. of Med., Tallahassee, FL; [4]Inst. for Pediatric Rare Dis., Tallahassee, FL

Abstract: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and a leading monogenic contributor to autism spectrum disorder. FXS is caused by transcriptional silencing of the *FMR1* gene and subsequent loss of fragile X messenger ribonucleoprotein (FMRP), a key regulator of synaptic development and plasticity. In addition to hallmark cognitive and behavioral symptoms, individuals with FXS frequently exhibit reduced motivation and impairments in reward processing—features that severely impact functional independence and quality of life. These symptoms likely stem from dysfunction in mesolimbic dopamine circuitry, which regulates motivation, reinforcement, and goal-directed behavior. Although dopaminergic dysfunction in the mesolimbic circuit has been implicated in FXS, the upstream regulators of this system remain unclear. The kappa opioid receptor (KOPR), a potent modulator of dopamine release, is enriched in the ventral tegmental area (VTA) and nucleus accumbens (NAc)—key nodes of the brain's reward system. Our recent studies in *Fmr1* knockout mice demonstrated upregulation of KOPR expression in the VTA and higher KOPR-induced cFOS expression in the cells of the NAc of *Fmr1* KO mice compared to wildtype controls. Furthermore, we found that systemic administration of selective KOPR antagonists restored motivated behaviors in the *Fmr1* KO mice, including nest-building and social

engagement. Collectively, these data show that KOPR expression and activity are upregulated in reward circuits in the *Fmr1* KO mice and that the upregulation contributes to deficits in motivated behavior. Ongoing studies aim to elucidate the molecular and circuit-level mechanisms by which KOPR signaling influences motivation to identify novel therapeutic strategies for FXS and related neurodevelopmental disorders.

Disclosures: M.X. Trupiano: None. **A.A. Nemec:** None. **C. Vied:** None. **P.G. Bhide:** None. **D.M. Mc Carthy:** None.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.16/B8

Topic: A.06. Developmental Disorders

Support: NARSAD YIG 28782
BB/V005405/1
Simons Initiative for the Developing Brain Start up funding

Title: Sensory-driven hypoplasticity in neocortical circuits of the Fragile X mouse model

Authors: P. C. KIND[1], A. CREED[1], E. HOEH[1], ***L. E. WILLIAMS**[2];
[1]Ctr. for Discovery Brain Sci., [2]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Sensory experience and learning are tightly linked to plasticity in neocortical circuits. Repetitive sensory stimulation—such as rhythmic whisker stimulation (RWS) at behaviorally relevant frequencies (~8 Hz)—can induce long-term potentiation (LTP) of excitatory synapses. Fragile X syndrome (FXS), a leading monogenically inherited form of intellectual disability, is associated with tactile hypersensitivity, where non-noxious touch can be aversive. To investigate how sensory-evoked plasticity is altered in FXS, we combined ex vivo whole-cell electrophysiology with in vivo two-photon calcium imaging of layer 2/3 (L2/3) pyramidal neurons (PNs) in adult male Fmr1-knockout ($Fmr1^{-/y}$) mice and wild-type ($Fmr1^{+/y}$) littermates. We found that plasticity induced by layer 4 repetitive electrical stimulation (L4-RES) and in vivo RWS was impaired in $Fmr1^{-/y}$ mice. L4-evoked subthreshold synaptic responses were hyperexcitable in L2/3 PNs, consistent with circuit-level disinhibition. Despite this, baseline intrinsic spiking evoked by current steps remained largely unchanged, although whisker-evoked activity appeared diminished *in vivo*. To probe potential mechanisms, we observed a shortening of the axon initial segment (AIS) in L2/3 in $Fmr1^{-/y}$ mice, and impaired sustained activity during RES and RWS periods—both of which may underlie the failure to induce plasticity. Together, these findings suggest that L2/3 PNs of S1 in $Fmr1^{-/y}$ mice are locked in a hypoplastic state, potentially due to disrupted inhibitory control and aberrant AIS plasticity, which may culminate in a failure of dendritic integration.

Disclosures: P.C. Kind: None. **A. Creed:** None. **E. Hoeh:** None. **L.E. Williams:** None.

Poster

PSTR371: Developmental Disorders

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Topic: A.06. Developmental Disorders

Support: NIH Grant NS118378
NARSAD Young Investigator Grant
Cleveland State University Startup grant
FRAXA fellowship

Title: Cofilin1 in extracellular vesicles regulates synaptic remodeling in recipient neurons.

Authors: *J. YAN;
Cleveland State Univ., Cleveland, OH

Abstract: Abnormal spine density and morphologies are linked to cognitive and behavioral deficits in autism and other neuropsychiatric disorders. Actin dynamics, mediated by actin depolymerization factor (ADF) and Cofilin1, are essential for synapse remodeling. This study explores Cofilin1-enriched extracellular vesicles (EVs) as a therapeutic delivery method to regulate spine morphology. Overexpression of Cofilin1 in neurons increased its content in secreted EVs without affecting EV production. These EVs enriched with Cofilin1 were taken up by recipient neurons, effectively modulating actin dynamics by reducing F-actin levels. In a Fragile X syndrome mouse model, delivery of EVs enriched with Cofilin1 corrected abnormal spine morphology and improved sensory processing and social behaviors. This indicates that Cofilin1-enriched EVs offer a promising therapeutic strategy for neuropsychiatric disorders by modulating synaptic actin dynamics. Our findings underscore the potential of EV-mediated delivery systems for targeted neurological disease interventions.

Disclosures: J. Yan: None.

Poster

PSTR371: Developmental Disorders

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Topic: A.06. Developmental Disorders

Support: HT94252410161 CDMRP award
R01NS137084 NIH award

Title: Investigating the role of astrocytes in deficient sleep in Fragile X syndrome mouse model

Authors: *A. ANDING[1], N. SANKAR[2], P. ZHONG[2], A. DUNAEVSKY[2];
[1]Genetics, Cell Biol. & Anat., [2]Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Clinical polysomnography studies have revealed children with FXS display rapid-eye movement (REM) sleep deficiency, and according to patient surveys, this altered sleep architecture often manifests as frequent nighttime awakenings and daytime drowsiness, among other behaviors. Sleep impairments in the *Fmr1* KO mouse model for FXS have been reported, however, the developmental trajectory of sleep architecture and mechanisms underlying sleep impairments remains poorly understood. Predominantly, neurons are implicated in the neuropathological etiology of FXS. However, FMRP is also expressed in astrocytes, and an increasing number of FXS animal studies implicate a role for astrocytic FMRP in FXS pathogenesis. Additionally, there is cumulative evidence that implicates astrocytic calcium (Ca^{2+}) activity in sleep physiology. We have reported abnormal increase in cytosolic Ca^{2+} signaling in FXS, however, whether altered cortical astrocyte Ca^{2+} events in FXS contribute to sleep impairments, has not been explored. In this study, we performed simultaneous cortical electroencephalography (EEG), electromyography (EMG) recordings and behavioral monitoring in *Fmr1* KO and astrocyte-deleted *Fmr1* cKO mice using telemetry devices to examine electrophysiological architecture associated with sleep and wake cycles in FXS and isolate the effect of astrocytic FMRP on sleep impairments. Recordings were collected from adolescence (P45) to adulthood (P150) to examine sleep architecture across development. We have found that across 24 hours, both younger and older *Fmr1* KO mice exhibit reduced sleep compared to controls, which is driven by a reduction in non-rapid eye movement (NREM) sleep. Further, *Fmr1* KO mice also display an increased number of microarousals across development, indicative of NREM sleep fragmentation. Preliminary data do not indicate reduced sleep in the *Fmr1* astrocyte cKO mice, however, these mice do exhibit an increased number of microarousals during NREM at 5 months of age. We have also performed sleep deprivation and recovery sleep analysis in 2-month and 5-month old *Fmr1* KO mice to assess sleep homeostasis. Preliminary data indicate this process is disrupted. In ongoing experiments, we are assessing astrocytic Ca^{2+} signaling in the cortex of awake and naturally sleeping *Fmr1* mutant mice while monitoring brain rhythms and behavior. We hypothesize that astrocyte Ca^{2+} signaling signatures associated with sleep phases will be altered in the FXS mice. Our findings will advance our understanding of sleep deficit behaviors and astrocyte involvement in aberrant sleep homeostasis in Fragile X syndrome.

Disclosures: A. Anding: None. **N. Sankar:** None. **P. Zhong:** None. **A. Dunaevsky:** None.

Poster

PSTR371: Developmental Disorders

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Topic: A.06. Developmental Disorders

Support: NIH grant R01MH136990
FRAXA fellowship
SFARI - Award: SFI-AN-AR-Pilot-00005618
Pershing Square Innovation Fund (PSIF)

Title: Early somatostatin neuron circuit reprogramming reverses sensory deficits in Fragile X mice

Authors: D. DUMONTIER[1], S. LIEBMAN[1], V. LE[2], S. GEORGE[3], D. VALDEMAR[4], L. VAN AELST[1], ***G. POUCHELON**[1];
[2]Neurosci., [3]Neuro, [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [4]Stony Brook Univ., Brentwood, NY

Abstract: Sensory dysfunction is a hallmark of neurodevelopmental disorders, yet the developmental mechanisms underlying these impairments remain poorly understood. Here, we show that in a mouse model of Fragile X syndrome (FXS), sensory deficits arise from the abnormal persistence of early thalamocortical inputs onto somatostatin (SST) inhibitory neurons, a circuit normally transient. In Fmr1 knockout mice, these aberrant inputs prevent the sequential bottom-up maturation of cortical networks, leading to FXS excitation/inhibition (E/I) imbalance and sensory dysfunctions. Targeted chemogenetic modulation of SST neurons in FXS mice during a critical postnatal window normalizes circuit progression and rescues mature cortical physiology and sensory-driven behaviors in adulthood. These findings identify early SST neuron circuit dynamics as a developmental gatekeeper of sensory cortical maturation and reveal a bottom-up circuit logic by which sensory phenotypes neurodevelopmental disorders may unfold.

Disclosures: D. Dumontier: None. **S. Liebman:** None. **V. Le:** None. **S. George:** None. **D. Valdemar:** None. **L. Van Aelst:** None. **G. Pouchelon:** None.

Poster

PSTR371: Developmental Disorders

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Program #/Poster #: PSTR371.20/B12

Topic: A.06. Developmental Disorders

Support: Simon STEM Scholars Program
Cold Spring Harbor Undergraduate Research Program

Title: Manipulating somatostatin neurons affect on the development of fragile x syndrome

Authors: *D. VALDEMAR[1,2], D. DUMONTIER[4], S. LIEBMAN[3], G. POUCHELON[2];
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Abstract: Deletion mutations in the FMR1 gene, on the X chromosome which codes for the FMRP protein causes the neurodevelopmental disease Fragile X syndrome (FXS). FMRP mutations lead to disruptions of brain functions, such as hypersensitivity, hyperactivity, and speech delay. These disruptions involve an imbalance in excitation and inhibition. Specifically the parvalbumin (PV) type of inhibitory neurons is impaired in FXS. However, we found that the somatostatin (SST) neurons, which are involved in transitory circuits during postnatal development, exhibit persistent circuits in a mouse model of FXS, the Fmr1 KO mice. We next tested the influence of these processes on PV neurons and brain activity disruptions associated with FXS. We first found that FMRP is expressed in SST neurons suggesting that SST neurons may be involved in the physiopathology of FXS. We next developed a chemogenetic strategy to regulate the aberrant SST neuron circuits and investigate their role in the etiology of FXS symptoms. We found that our strategy specifically targeted SST neurons and restored their early circuits. This led to the restoration of PV density and other circuit motifs, suggesting SST neurons are regulators of developing cortical circuits, which deficits are associated with FXS.

Disclosures: D. Valdemar: None. **D. Dumontier:** None. **S. Liebman:** None. **G. Pouchelon:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.21/B13

Topic: A.06. Developmental Disorders

Title: Fmrp regulation of molecular signatures of neuron subtypes in the mouse spiral ganglion

Authors: *D. ALQURAISH[1], M. LANA[2], B. DONALDSON[2], Y. WANG[3];
[1]Program in Neuroscience, Dept. of Biomed. Sciences, Col. of Med., [2]Dept. of Biomed. Sciences, Col. of Med., [3]Dept. of Biomed. Sci., Florida State Univ., Tallahassee, FL

Abstract: Spiral ganglion neurons (SGNs) are the first to fire action potentials in the auditory pathway, transmitting sound information to the brain. In mature mice, SGNs diversify into four subtypes IA, IB, IC, and II, each with distinct connections, electrophysiology, and gene expression. Type I SGNs, which connect cochlea with the brain, show graded expression of markers such as calretinin (CR) in IA and IB, and Pou4f1 and Lypd1 in IC. These molecular differences enable SGNs to encode a wide range of sounds. Previous studies indicate that SGN subtype identity is gradually refined during development and requires hair cell-driven activity. However, the molecular pathways underlying this process remain unclear. Fragile X messenger ribonucleoprotein 1 (FMRP), encoded by *Fmr1*, is an RNA-binding protein that regulates neuronal maturation and circuit formation, often via activity-dependent processes. We hypothesize that FMRP plays a regulatory role in SGN subtype refinement. To test this, we compared the development of SGN subtypes in wild-type (WT) and *Fmr1* knockout (KO) mice at three ages: early development (postnatal day 14, P14), young adulthood (2 months old), and

aging (10-12 months old). We confirmed the varied expression of CR in WT mice and identify NeuN (encoded by *Rbfox3*) as a novel subtype marker, displaying a complementary expression pattern to CR. Neurons with high CR had low NeuN, and vice versa. In WT mice, cellular CR immunoreactivity, measured by corrected total cellular fluorescence, increased in the aged group. This increase aligns with previous reports of larger proportions of IA and IB subtypes during aging. This increase, however, was diminished in *Fmr1* KO mice, suggesting a delay or reduction in age-related SGN subtype deterioration. Conversely, cellular NeuN immunoreactivity declined in the aged WT group, consistent with a smaller proportion of IC neurons. The complementary relationship between CR and NeuN was preserved in *Fmr1* KO mice; however, NeuN levels were elevated at all three ages compared to WT, suggesting a shift toward increased IC-type identity. These changes in CR and NeuN expression indicate disrupted development and maintenance of SGN molecular identity in the absence of FMRP, which may alter subtype composition. Our findings identify FMRP as a novel regulator in the maturation of the mammalian spiral ganglion and provide insights into the mechanistic understanding of auditory deficits in diseases associated with FMRP dysfunction, such as Fragile X syndrome.

Disclosures: D. Alquraish: None. **M. Lana:** None. **B. Donaldson:** None. **Y. Wang:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.22/B14

Topic: A.06. Developmental Disorders

Title: Probabilistic reversal learning in mouse models of Fragile-X Syndrome

Authors: *C. SCARAMELLA[1], K. M. HUBER[2], K. A. RAZAK[3];
[1]Neurosci., Univ. of California, Riverside, Riverside, CA; [2]Neurosci., UT Southwestern Med. Ctr., Dallas, TX; [3]Psychology, Univ. California, Riverside, Riverside, CA

Abstract: Behavioral flexibility is the ability for an animal to adapt to new rules or changing environments, a skill that can be crucial for survival. Deficits in behavioral flexibility are frequent, pronounced, and among the most functionally impairing symptoms in autism spectrum disorders (ASD), often manifesting as repetitive behaviors including perseveration and rigid behavioral preferences. Fragile X Syndrome (FXS) is the leading known monogenetic cause of intellectual disability, with symptoms that overlap with ASD. FXS is the result of transcriptional silencing of the *Fmr1* gene, which leads to a lack of fragile X messenger ribonucleoprotein (FMRP). *Fmr1* knock-out (KO) mouse models of this disorder show consistently similar phenotypes to those seen in humans with FXS. Recently, our lab demonstrated a robust deficit in male C57Bl/6 *Fmr1*-KO mice to update their decision-making in an 80:20 probabilistic reversal learning (PRL) paradigm, a phenotype that has also been seen in clinical settings with a similar task. However, it remains unclear how FMRP levels contribute to this phenotype throughout development and whether FMRP re-expression in the adult brain is sufficient to reverse this

deficit. Thus, we crossed *Fmr1*-Neo$^{fl/+}$ mice (cON) with a tamoxifen-inducible CaMKII-Cre line to achieve FMRP re-expression in the excitatory neurons of the forebrain from two ages in development (P14, P60). After undergoing treatment with tamoxifen to re-express FMRP 15 days prior, adult *Fmr1*-KO mice continued to make more perseverative errors thus taking significantly more trials to learn the reversal task, however the cON mice no longer demonstrated this phenotype. This indicates that even adult reinstatement of FMRP is sufficient to improve behavioral flexibility. Unlike the adults, adolescent *Fmr1*-KO mice did not show any deficits in reversal learning when tested ~p40. Finally, we are utilizing adult male mice in which cannabinoid 1 receptors (CB1Rs) are selectively reduced in the inhibitory neurons of both wild-type and *Fmr1*-KO mice. We expect that heterozygous deletion of the *CNR1* gene in inhibitory neurons will improve reversal learning behavior in the *Fmr1*-KO/VGAT-Cnr1$^{+/-}$ mice due to normalization of inhibitory circuit control. The results of these studies have major implications in identifying potential avenues and timing of treatment for cognitive flexibility in humans with FXS and related spectrum disorders.

Disclosures: C. Scaramella: None. **K.M. Huber:** None. **K.A. Razak:** None.

Poster

PSTR371: Developmental Disorders

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.23/B15

Topic: A.06. Developmental Disorders

Support: Welch Foundation Grant C-2199

Title: Elucidating the post-transcriptional control of DNMT3A in brain development

Authors: *A. ESCOBAR[1], S. E. PATINO HERNANDEZ[2], L. LAVERY[3];
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Abstract: Mammalian brain development encompasses an orchestrated sequence of events that regulate gene expression over distinct time points. Epigenetic factors serve as critical regulators in this process, ensuring proper gene expression. In mammals, DNA methylation is an inheritable epigenetic modification that plays a role in various biological processes. DNA methyltransferase 3 alpha (Dnmt3a) is a DNA methyltransferase responsible for adding methyl groups to unmethylated cytosines, thus establishing DNA methylation patterns in the genome. Previous knockout mouse studies have demonstrated the indispensability of Dnmt3a in infant development. Additionally, recent discoveries have revealed DNMT3A mutations to be causal for several developmental and learning disorders. Furthermore, studies have provided insight into the expression patterns of two distinct isoforms of DNMT3A, which exhibit differential expression from stem cells to somatic tissues. This has shown that the isoform DNMT3A1 is exclusively responsible for the establishment of mCH methylation in mature neurons. Mouse

studies have demonstrated that ablation of this isoform leads to neuromuscular defects and a short life span. Despite its relevance in brain maturation, the basis of the Dnmt3a-dependent methylation mechanism remains unknown. Therefore, there is a pressing need to understand the regulatory mechanism and function underlying DNMT3A to ensure proper gene regulation during mammalian development. To further elucidate the constituents of this regulatory process, we explored the influence of post-transcriptional factors that govern DNMT3A. We have characterized the role of relevant histone modifications previously associated with DNMT3A-catalyzing activity in mouse striatal tissue across critical development time points. We have identified a set of histone marks that co-localize with DNMT3A-dependent mCH methylation at gene bodies of neuronal genes, suggesting a coordinated mechanism that facilitates mCH deposition. Using a combination of CUT&RUN profiling and transcriptomic analysis, we provide evidence that these factors act in concert to regulate DNMT3A recruitment, activity, and downstream transcriptional outcomes during neuronal maturation.

Disclosures: A. Escobar: None. **S.E. Patino Hernandez:** None. **L. Lavery:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.01/B16

Topic: A.08. Adolescent Development

Support: NIH Grant from NINDS R25NS130965
NIH Grants U01DA041048, U01DA050989, U01DA051016, U01DA041022, U01DA051018, U01DA051037, U01DA050987, U01DA041174, U01DA041106, U01DA041117, U01DA041028, U01DA041134, U01DA050988, U01DA051039, U01DA041156, U01DA041025, U01DA041120, U01DA051038, U01DA

Title: Longitudinal Relationships Between Putative Neuroinflammation, BMI, and Depressive Symptoms in Youth

Authors: *A. CROSBY[1], Z. LI[2], M. JANSEN[4], T. HERSHEY[3];
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Abstract: Obesity and depression rates in youth have risen significantly over the past decade. Youth with elevated body mass index (BMI) are more likely to experience future depressive symptoms, yet the biological mechanisms driving this association remain unclear. Human imaging studies suggest that neuroinflammation may link higher BMI to depression. This study examined whether restriction spectrum imaging (RSI) markers of neuroinflammation mediate the relationship between BMI and depressive symptoms in youth over 2 years. Data were drawn

from the Adolescent Brain Cognitive Development (ABCD) Study (release 5.1). We analyzed data from 9,188 children (baseline mean age = 9.9 years, 51% male), with complete data at baseline and 2-year follow-up. Depressive symptoms were assessed using raw scores from the Child Behavior Checklist DSM-5 Depression and the Withdrawn/Depressed Syndrome subscales. Neuroinflammation was measured using diffusion-weighted magnetic resonance imaging and analyzed with RSI. We used the restricted normalized isotropic (RNI) diffusion metric to indicate degree of cellularity, with higher RNI values interpreted as greater neuroinflammation. We focused on five brain regions with prior evidence implicating them in depression, and control regions not known to be associated with depression were used to assess the anatomical specificity of this pathway. Linear mixed-effects models were used to examine associations between baseline BMI, baseline RSI, and 2-year depressive symptoms, controlling for sex, age, income, head motion in the scanner, and intracranial volume. Structural equation modeling tested whether baseline RSI values mediated the association between baseline BMI and depressive symptoms at year two. Consistent with previous research, higher baseline BMI predicted increased depressive symptoms at follow-up (e.g., DSM-5 scale: $\beta = 0.04$, $p < .001$). BMI was also positively associated with RNI values in depression related regions (e.g., left amygdala: $\beta = 0.03$, $p = .001$; left hippocampus: $\beta = 0.05$, $p = .001$) and control regions (e.g., left cerebellar white matter: $\beta = 0.07$, $p = .001$. However, mediation models showed non-significant indirect effects ($p > .05$), suggesting RSI-measured neuroinflammation did not mediate the BMI-depression relationship. Neuroinflammation, as measured by RSI, does not explain the pathway from obesity to depression in youth. Future studies should investigate other possible mediators, including social stigma, caregiver-child conflict, impaired reward processing, or HPA-axis dysregulation.

Disclosures: A. Crosby: None. **Z. Li:** None. **M. Jansen:** None. **T. Hershey:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.02/B17

Topic: A.08. Adolescent Development

Support: U01DA041048
U01DA050989
U01DA051016

Title: Brain morphological pattern predicts the existence of transdiagnostic psychiatric disorders in preadolescents

Authors: *N. KUANG[1], Y. YANG[2];
[1]NIH, BALTIMORE, MD; [2]Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: Cognitive function and general psychopathology are two core behavioral dimensions whose underlying neurodevelopmental substrates remain incompletely understood. In a large pediatric cohort, we employed multivariate latent-variable modeling to examine associations between cortical morphometric measures and multidomain assessments of cognition and psychopathology at baseline and again two years later. We identified a robust latent morphological pattern that was positively associated with cognitive performance and inversely associated with psychopathology across traditional diagnostic boundaries. Importantly, the expression of this pattern remained stable at follow-up. Moreover, lower pattern scores at baseline predicted greater psychiatric comorbidity over the two-year interval, whereas higher scores predicted resilience, with individuals maintaining healthy status over time. These results suggest that a shared cortical structural substrate reflects a vulnerability-resilience continuum for youth mental health. By highlighting a transdiagnostic morphological signature that is evident before and after the onset of clinical symptoms, our findings underscore the potential utility of morphology-informed approaches for early screening and intervention in children and adolescents.

Disclosures: N. kuang: None. **Y. Yang:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.03/B18

Topic: A.08. Adolescent Development

Support: NIH Grant R01 MH125479
NIH Grant R01 EB008374
NIH Grant R01 MH133836
NIH Grant R01 EB035160

Title: Early Structure-Function Coupling and Its Association with Myelination and EI Balance

Authors: *J. MAO[1], K. HUYNH[2,3], K. THUNG[2,3], S. AHMAD[2,3], P.-T. YAP[2,3];
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Abstract: Understanding how brain structure supports function is essential for uncovering the neural basis of cognition (Baum., 2020). While this relationship is well-characterized in adults, it remains poorly understood in infancy due to limited longitudinal data and reliance on coarse, region-based approaches. Here, we use a fine-scale, vertex-wise analysis to capture developmental changes during early childhood, offering insight into how brain architecture supports coordinated activity.Using structural, functional, and diffusion MRI from 380 scans spanning birth to five years of age (Howell., 2019), we computed vertex-wise measures of

structure-function coupling (SFC; derived from functional connectivity and tractography-based structural connectivity), the Hurst exponent (H; a proxy for synaptic excitation-inhibition balance), and myelin content (T1w/T2w ratio) (Fotiadis., 2023). Developmental trajectories of SFC, H, and myelination were modeled using generalized additive mixed models (GAMMs) (Taylor IV., 2024).

SFC is strongest in regions that support basic functions: visual and somatomotor networks. SFC is weaker in higher-order association areas: the default mode, ventral attention, and limbic networks (Buckner., 2013). Generally, SFC increases with age in most networks, similar to the trend of myelination; except for the frontoparietal network where SFC decreases while myelination increases.

H decreased with age, indicating a developmental shift toward more complex BOLD dynamics (Churchill., 2016). H also declined along the unimodal-to-transmodal gradient, with the highest values in somatomotor areas and lowest in limbic regions.SFC correlated positively with both H ($r = 0.57$, $p < 0.0001$) and myelin ($r = 0.67$, $p < 0.0001$), suggesting that greater structure-function alignment is linked to increased microstructural maturation and more stable neural signal dynamics.

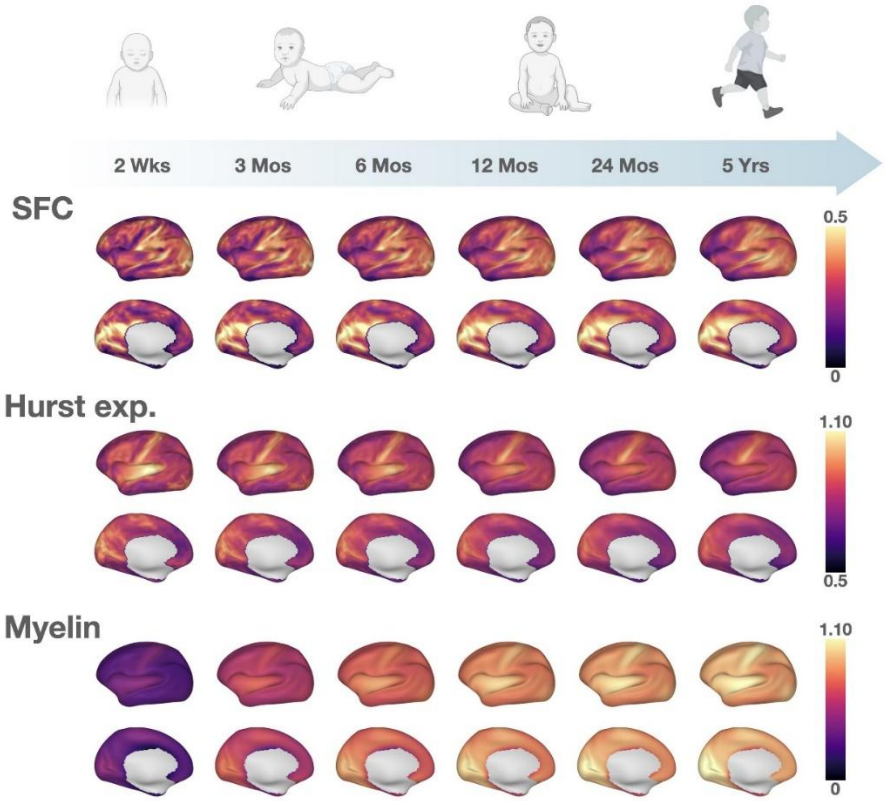

Figure 1: Developmental trajectories of structural–functional coupling (SFC), Hurst exponent, and myelination across infancy and early childhood. Vertex level surface plots showing GAMM-fitted developmental trajectories, from 2 weeks to 5 years of age, visualized at selected timepoints corresponding to key developmental milestones.

Disclosures: J. Mao: None. **K. Huynh:** None. **K. Thung:** None. **S. Ahmad:** None. **P. Yap:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.04/B19

Topic: A.08. Adolescent Development

Support: NSF CREST DMAP HDR #2112455

Title: The role of genetic vulnerability and adversity in problematic adolescent behavior and neuronal development

Authors: *D. JENSEN;
Translational Res. in Neuroimaging and Data Sci., Georgia State Univ., Atlanta, GA

Abstract: Adolescence is a crucial period where the interplay between environment and genetics informs behavioral development. Disruption of this process can lead to the emergence of problematic behaviors such as internalizing and externalizing. Studies show these behaviors share common genetic vulnerability, quantified as polygenic risk scores (PRS) and are associated with adverse childhood experiences (ACEs). We lack understanding of the dual influence of these factors on co-developmental trajectories of internalizing and externalizing behaviors and how these impact neuronal development.A parallel process latent class growth analysis, using the Adolescent Brain Cognitive Development cohort (N=11k, 4 timepoints, aged 9-12), was applied to model the dual trajectories. Behavior scores were used for the observations, level of ACEs and four PRS measures were included as predictors. Fractional anisotropy (FA) measures were calculated from diffusion images and were included in a multivariate analysis of covariance (MANCOVA) to explore differences in FA based on ppLCGA group membership.The fit indices showed a 4-class model was best fit. These subgroups were: Low Level (86%), Increasing (4%), Decreasing (5%), and High Level (5%) behaviors. Higher general genetic risk for mental illness was associated with rates of increasing internalizing behaviors ($t=2.066$, $p<0.039$), while higher genetic risk for ADHD and ASD was associated with higher starts of the externalizing trajectories ($t=4.629$, $p<0.00001$). Higher genetic risk for alcohol dependency was associated with the increases in internalizing ($t=2.506$, $p<0.012$) and externalizing ($t=2.392$, $p<0.017$). Lower ACEs were related lower levels of internalizing ($t=-3.998$, $p<0.0001$) and externalizing ($t=-2.428$, $p<0.015$). The MANCOVA results revealed group differences ($f = 1.56$, $p<0.0000001$), highlighting increased FA for subjects with high levels of problematic behaviors in regions such as the cerebellum, superior longitudinal fasciculus, and splenium.While a large percentage of this cohort exhibited low level behaviors across early adolescence, high levels of genetic risk for various mental illnesses in childhood, as well as high levels of adversity, were strongly associated with membership in the high level of problematic behaviors group. This same

group showed accelerated development of their white matter in regions of the brain responsible for emotional regulation, attention, and executive function compared to their peers in other behavior groups. These findings further support the stress acceleration hypothesis of advanced brain development in the face of genetic vulnerability and adversity.

Disclosures: D. Jensen: None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.05/B20

Topic: A.08. Adolescent Development

Support: CONACYT grant CF-2019-6390
DGAPA-PAPIIT-UNAM IN208622
DGAPA-PAPIIT-UNAM IN212624

Title: Childhood trauma and amygdalar volume: a twin study

Authors: *E. ZALDÍVAR MORALES[1], D. RAMÍREZ GONZÁLEZ[3], G. L. LICEA HAQUET[6], I. ESPINOSA MÉNDEZ[7], G. ROBLES RODRÍGUEZ[8], A. SOLARI[4], T. V. ROMAN-LOPEZ[9], E. PASAYE[10], I. C. SÁNCHEZ[11], A. MEDINA-RIVERA[12], M. E. RENTERIA[13], A. E. RUIZ-CONTRERAS[2], S. ALCAUTER[5];
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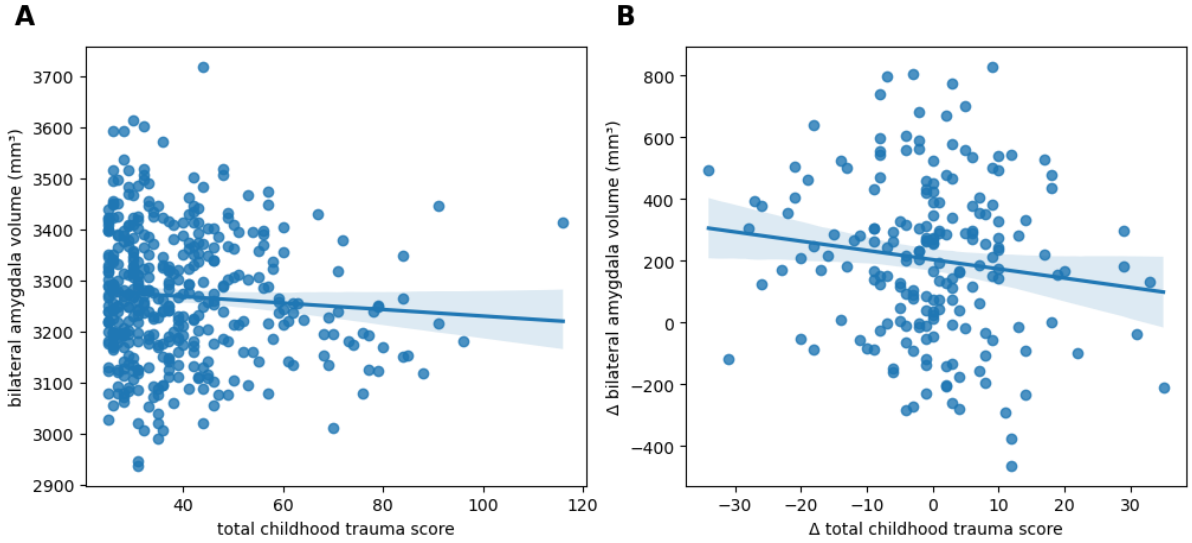
Abstract: Childhood trauma has been associated with structural differences in limbic regions such as the amygdala, but this association could arise due to other genetic or environmental factors. Here, we leveraged a twin design to examine whether differences in trauma exposure correlate with volumetric differences in the amygdala. Data was drawn from the Mexican Twin Registry (TwinsMX) and included 416 participants (32.16 +/- 9.63 years old, 314 Female), including 30 unpaired twins and 193 twin pairs, (131 monozygotic and 62 dizygotic) who completed the Childhood Trauma Questionnaire-Short Form (CTQ-SF) and underwent brain

MRI (3T scanner), including a high-resolution T1w image. We used two approaches to analyze the data. In our subject-level analysis, we used a mixed model to examine the association between the bilateral volume of the amygdalae or the hippocampi and the childhood trauma score, where the intracranial volume [ICV] was used as a covariate; while controlling for age, sex, zygosity, intracranial volume, and including family as a random intercept. We observed a significant negative association between the childhood trauma score and the total amygdala volume (β=-2.486, p=0.026. Figure 1A), but not with the hippocampi (β=-2.043, p=0.319). In our sibling-level approach, we analyzed the association between each sibling's difference in childhood trauma scores and the difference in total amygdala volume. We observed a marginally non-significant negative association when we included all twin pairs (β=-2.9995, p=0.067. Figure 1B) and when we only included MZ pairs·(β=-3.1432, p=0.087).These findings support an environmentally shared feature between childhood trauma and amygdala morphology. The twin difference design strengthens the causal interpretation and underscores the importance of early interventions to mitigate long-term neurobiological impacts of childhood adversity.



Disclosures: E. Zaldívar Morales: None. **D. Ramírez González:** None. **G.L. Licea Haquet:** None. **I. Espinosa Méndez:** None. **G. Robles Rodríguez:** None. **A. Solari:** None. **T.V. Roman-Lopez:** None. **E. Pasaye:** None. **I.C. Sánchez:** None. **A. Medina-Rivera:** None. **M.E. Renteria:** None. **A.E. Ruiz-Contreras:** None. **S. Alcauter:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.06/B21

Topic: A.08. Adolescent Development

Support: U01DA041089 (PI: Tapert)

Title: Physical activity and structural brain volume among mid-adolescents: comparison between metabolic activity and sports involvement

Authors: *J. K. LYMAN[1], R. SULLIVAN[1], A. WALLACE[1], N. WADE[1], K. E. COURTNEY[3], J. JACOBUS[1], S. F. TAPERT[2];
[1]Psychiatry, Univ. of California San Diego, San Diego, CA; [2]Psychiatry, Univ. of California San Diego, La Jolla, CA; [3]UCLA, Los Angeles, CA.

Abstract: Physical activity plays a critical role in adolescent structural neurodevelopment; however, its impact on specific brain regions remains less understood. Metabolic equivalent of task (MET) scores presents a quantifiable avenue to address this gap. METs are a standardized numerical index of physical activity intensity which accounts for both type and duration of activity, offering a more nuanced alternative to frequency of activity metrics. Despite their widespread use in exercise science and epidemiology, METs remain underutilized in developmental imaging research. This study investigates the associations between the past-year total number of sports participated in and MET scores and structural brain volume in adolescents (ages 13-15) from the Adolescent Brain Cognitive Development (ABCD) Study. Participants (N=3,727) were selected who engaged ≥1 past-year sport and had high-quality structural MRI data. A series of linear mixed-effects models examined associations between physical activity and brain structure volume across all 84 Desikan volumetric regions of interest. Models predicted either (1) total METs or (2) total sports, while controlling for age, estimated total intracranial volume, household income, and variance attributable to differing MRI scanner and family relatedness. False-discovery rate corrections were applied across models. Chi-square testing of fit indices (i.e., residual deviance test) compared empty versus full models for activity-related predictors. Analyses revealed significant positive associations between MET scores and the right paracentral gyrus [$t(3155) = 3.6$, $pFDR = 0.03$], such that greater levels of physical activity, indicated by higher MET scores, were associated with increased volume in this motor-related brain region. No significant associations were observed ($pFDR \geq .14$) in models using the total number of sports as the physical activity measure. Models incorporating METs (vs. total sports) had significantly improved model fit indices ($\chi2=10.2$, $p=.001$). The paracentral gyrus plays a role in motor execution and planning, suggesting that higher-intensity physical activity may relate to neural differences underlying sensorimotor control. These findings underscore the potential of METs in capturing meaningful physical activity variation in brain morphometry and provide preliminary evidence that increased physical activity may be associated with region-specific variation during adolescence. These findings warrant longitudinal follow-up to examine directionality in how changes in physical activity may shape brain structure across adolescent development.

Disclosures: J.K. Lyman: None. **R. Sullivan:** None. **J. Jacobus:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.07/B22

Topic: A.08. Adolescent Development

Support: Bezos Family Foundation

Title: Accelerated maturation of brain circuits and of executive function in adolescents who experienced the COVID-19 pandemic lockdown

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

Abstract: The COVID-19 pandemic lockdown disrupted adolescents' daily routines, social interactions, and education during an important neurodevelopmental period, which prompted investigations into its effects on behavior and brain development. Our previous study revealed accelerated gray matter maturation following the pandemic lockdown, which was more pronounced in females. However, less is known about changes in white matter development, or about changes in the development of executive function (EF), a key cognitive domain that develops throughout adolescence. The current study examined whether white matter development was altered by the lockdown, whether effects varied by sex, and whether development of EF was affected.

Longitudinal cognitive and diffusion-weighted imaging (DWI) data were collected at two timepoints: pre-pandemic (2018) and post-lockdown (2021-2022). The pre-COVID sample consisted of 164 subjects (81F) aged 9-17 years. The post-COVID sample consisted of 137 subjects (70F) aged 12-20 years. EF scores on the Flanker and Dimensional Change Card Sort (DCCS) were collected. Fractional anisotropy (FA) and mean diffusivity (MD) were calculated from the DWI data for 16 white matter tracts. For both the cognitive and brain imaging measures, normative models were built from the pre-pandemic sample and used to quantify deviations from typical development in the post-pandemic sample.

Post-COVID performance on the Flanker and DCCS tasks was significantly higher than predicted by the normative models, suggesting accelerated development in these executive control functions ($p<0.05$; all stats corrected unless noted). Post-COVID MD values were significantly lower than predicted by the normative models in 10 tracts in females, and in 2 tracts for males, representing acceleration of development. Post-COVID FA values were significantly higher than predicted by the normative models in 2 tracts in females (representing acceleration), and lower than predicted in 2 tracts in males ($p<0.05$). Significant sex differences in post-COVID FA were observed in 4 of the 16 tracts ($p<0.05$). Exploratory analyses revealed a significant correlation (uncorrected $p = 0.011$) between post-COVID Flanker performance and MD in the corpus callosum major, suggesting a possible relationship between cognitive and white matter changes.

These findings suggest that pandemic-related disruptions were associated with accelerated white matter maturation in both sexes, along with unexpectedly advanced performance on two

executive function tasks. Together, they indicate that the disruptions may have accelerated brain maturation in tandem with specific aspects of cognition.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.08/B23

Topic: A.08. Adolescent Development

Support: NIH Grant 5R01DA053301-04

Title: Mental health subtypes reveal brain structural markers of vulnerability and resilience

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Abstract: What structural brain features are associated with psychological risk and resilience in adolescents, especially under different socioeconomic environments? Adolescent mental health is shaped by both neurobiological and environmental factors. Understanding how brain structure supports resilience under conditions of adversity—such as environments of limited financial resources—can inform prevention and intervention strategies. Using baseline data from the Adolescent Brain Cognitive Development (ABCD) study (N = 10,842), we applied k-means clustering to four key variables: internalizing symptoms, externalizing symptoms, somatic symptoms, psychosis-like experiences, and family income. Five robust subgroups emerged, confirmed via 100× bootstrapping (Jaccard indices > 0.84), with interpretable profiles: • G1 - mixed SES; high PQ-BC score (psychosis severity) and moderate CBCL internalizing, externalizing, and somatic symptoms (n = 736) • G2 - mixed SES; high CBCL symptoms, low PQ-BC severity (n = 1,547) • G3 - low SES; low symptoms across all domains (n = 1,606) • G4 - high SES; moderate CBCL symptoms, low PQ-BC severity (n = 3,164) • G5 - high SES; low symptoms (n = 3,789). We validated the clustering by examining longitudinal trajectories (4 follow-up waves) in externalizing and externalizing and prodromal psychosis symptom measures. Clusters showed persistent within-group behavioral patterns across time, indicating meaningful psychological subtypes. Cognition scores followed a monotonic pattern aligned with SES and mental health (G1<G2<G3<G4<G5), while impulsivity symptoms showed the inverse (G1>G2>G3>G4>G5). Notably, clusters G3 and G5 exhibited the lowest exposure risk, while G4—high SES, moderate non-psychosis risk—showed elevated substance access. Substance accessibility scores were significantly correlated with both internalizing ($r = 0.10$) and externalizing ($r = 0.12$) scores (FDR-corrected, $p < 0.001$). Controlling for SES, G1 and G2

exhibited significantly reduced cortical area and volume in multiple areas including orbitofrontal, insular, and anterior cingulate regions—key nodes for introspection and emotional regulation—compared to G3, G4 and G5. In contrast, G3 (low-SES, low-risk) showed significantly deeper left superior frontal sulci (a putative marker of neurodevelopmental efficiency) relative to all other groups, possibly reflecting a compensatory strategy under socioeconomic constraint. Our results identify distinct behavioral and structural profiles in adolescents. These findings underscore the interaction between environmental exposure, brain development, and psychological outcomes in youth.

Disclosures: Y. Wang: None. **A.J. Holmes:** None. **D. Bzdok:** None. **S. Yip:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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NIH Grant R01AG076838
NIH Grant U24DA055330

Title: Brain Iron Accumulation and ADHD Risk in Adolescence

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Abstract: Adolescence is a period of profound neurobiological and behavioral change, during which the brain undergoes significant maturation, including the refinement of dopaminergic pathways that are critical for cognitive and emotional regulation. This developmental window also coincides with the emergence of neuropsychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD). MRI-based estimates of brain iron concentration suggest that deep gray matter structures, particularly regions rich in dopaminergic innervation, show marked increases in iron levels during adolescence. Given the role of iron in dopamine metabolism and neurotransmission, alterations in iron regulation may contribute to the pathophysiology of ADHD. To explore this, we applied a newly developed multivariate neuroimaging biomarker of central iron dysregulation, known as the Hemochromatosis Brain (HB), to evaluate whether iron dysregulation is linked to an increased risk of ADHD in

adolescents from the ABCD Study. The HB biomarker aggregates iron-related effects across the cerebellum and basal ganglia. It was pre-trained on T2-weighted MRI brain scans from 960 individuals in the UK Biobank to predict C282Y genotype status — a mutation associated with systemic iron dysregulation and the iron overload disorder, Hemochromatosis — achieving high accuracy (AUC = 0.86). Using T2-weighted MRI brain scans we calculated HB scores, indicative of central iron dysregulation, for 9959 adolescents (aged 9-11 at enrollment) from the study. Across timepoints HB scores increased as the sample aged indicating higher iron concentration through development with age accounting for 16% of variance from ages 9-15 ($p<10^{-100}$) - consistent with previous literature and known dopaminergic maturation. Surprisingly, at baseline (9-11 years old) we also found this HB score specifically predicted variability inattention subscales of the child behavior checklist including ADHD symptoms ($t(9959)=3.38$, $p=7.32x10^{-4}$) and attention problems($t(9959)=2.68$, $p=7.43x10^{-3}$) - with the positive direction indicative higher central iron levels. These findings provide novel mechanistic insight, which indicates a neurobiological phase advancement that is associated with ADHD risk. This may represent an early maladaptive maturation profile of the dopaminergic system deepening our understanding of normative and disrupted neural development.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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Topic: A.08. Adolescent Development

Support: NIH R21HD108757
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Title: Adolescent neural activity during associative memory encoding varies with dietary nutrients

Authors: *C. T. EICKSTEAD[1], A. BOLARAM[2], K. S. MOHANKUMAR[1], D. K. GREY[2], C. A. ORIHUELA[1], R. A. EVANS[1], S. A. MRUG[1], D. C. KNIGHT[3];
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Abstract: The dietary nutrients we consume may impact memory encoding as well as the neural function that supports it. For example, high levels of certain micro- and macronutrients (e.g., iron, zinc) may improve memory, while high levels of other dietary nutrients (e.g., saturated fats, added sugars) may worsen memory. Associative memory encoding is mediated by the prefrontal cortex (PFC), posterior cingulate cortex, and medial temporal lobe. Therefore, this project

investigated the relationship between adolescent nutrient intake and neural activity during an associative verbal memory encoding task. Associative memory was assessed in 37 adolescents (mean age = 15.22 years, standard deviation = 0.85) using a Verbal Paired Associates (VPA) task during functional magnetic resonance imaging (fMRI). Specifically, paired associate and single word trials were presented during fMRI. Paired associate trials consisted of two real words presented together, while single word trials consisted of one real word and a random letter string. Participants were asked to remember paired associate words, but ignore single words, for post-scan memory tests. Specifically, participants were asked to indicate whether each individual word or word pair was presented during fMRI. Adolescent nutrient intake was collected using a 24-hour diet recall and Nutritionist Pro software. Linear mixed effects (LME) models were used to assess whether neural activity during the VPA task (i.e., paired associate vs single words and remembered vs forgotten word pairs) varied with dietary nutrients. Age, sex, race, total kilocalories, socioeconomic status, body mass index, and VPA accuracy were included as covariates in all LME models. Dorsolateral PFC, dorsomedial PFC, ventrolateral PFC, parahippocampal gyrus, and hippocampal activity was greater during paired than single words ($P^{FWE} < 0.05$). Further, dorsolateral PFC activity was greater during remembered than forgotten word pairs ($P^{FWE} < 0.05$). We also found that memory-related PFC (i.e., dorsolateral, dorsomedial, and ventrolateral) and hippocampal activity varied with dietary nutrients (i.e., iron, zinc, and added sugars; $P^{FWE} < 0.05$). These results suggest that dietary nutrients (i.e., iron, zinc, and added sugars) may affect verbal associative memory-related brain function. These findings may have important implications for understanding the interrelationships between adolescent diet, neural activity, and memory processes.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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Program #/Poster #: PSTR372.11/B26

Topic: A.08. Adolescent Development

Support: R01DA053301

Title: Multidimensional components of impulsivity during early adolescence: Relationships with brain networks

Authors: *A. CHENG[1], S. RILEY[2], R. KOHLER[2], F. UMUTONI[2], M. N. POTENZA[3], S. D. LICHENSTEIN[2], A. J. HOLMES[4], D. BZDOK[5], S. YIP[2];
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Abstract: Impulsivity is a multifaceted construct that typically increases during adolescence and is implicated in risk for substance use disorders that develop later in life. Here, we take a multivariate approach to identify latent dimensions of impulsivity, broadly defined, among youth enrolled in the Adolescent Brain and Cognitive Development (ABCD) study and explore associations with individual differences in demographics, substance-use initiation and canonical resting state networks (N=11,872, ages ~9-10). Using principal component analysis, we identified eight latent impulsivity dimensions, the top three of which together accounted for the majority of the variance across all impulsivity assessments. The first principal component (PC1) was a general impulsivity factor that mapped onto all impulsivity-related assessments. PC2 mapped onto a 'mixed' impulsivity style related to both poorer, less attentive performance on the SST and decreased delay discounting. PC3 linked externalizing behaviors across multiple measures with indices of delay discounting, making delay discounting the only impulsivity-related assessment to load on all three of the top PCs. Multiple impulsivity PCs were significantly associated with subsequent initiation of alcohol and cannabis use. Finally, we found both cross-sectional and longitudinal associations between the PCs and functional connectivity between and within frontoparietal, cingulo-opercular, and default mode networks. These data provide a critical empirical baseline for how facets of impulsivity covary in early adolescence which may be tracked through future waves of ABCD data, enabling longitudinal elucidation of how dimensions of impulsivity interact with other individual and environmental factors to influence risk for substance use later in life.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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Program #/Poster #: PSTR372.12/B27

Topic: A.08. Adolescent Development

Title: Decoding Preadolescent Anxiety with Machine Learning Insights from Neuroimaging Data

Authors: O. CARRIOLI[1], A. CAMASSA[2], D. GREENE[3], T. J. SEJNOWSKI[5], *J. G. FLEISCHER[4];
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Abstract: Anxiety disorders are highly prevalent in adolescents, yet early identification remains limited by subjective symptom reports and diagnostic ambiguity. This project examines whether brain activation during an emotion and working memory task can differentiate preadolescents

with anxiety from control subjects. Using baseline data from the Adolescent Brain Cognitive Development Study, we trained a Random Forest classifier on brain regions activation coefficients from the Emotional N-Back paradigm in a carefully balanced sample of 515 participants aged 9 to 10. The dataset was stratified by scanner type, site, sex, and diagnostic group to minimize confounding effects, and medication usage was included as an input feature to account for treatment-related differences. Classification performance was highest for the 2-back versus 0-back contrast condition, reaching 0.74 accuracy and a 0.78 Area Under the Receiving Operator Characteristic curve on the held-out test set. Permutation testing confirmed these results as statistically significant. Additionally, feature importance analysis identified the five most predictive brain regions as the medial orbitofrontal cortex, supramarginal gyrus, middle temporal gyrus, temporal pole, and postcentral gyrus. ADHD medication use also emerged as a strong predictor. These results suggest that brain activation under high working memory load captures meaningful differences in anxious youth. While medication effects remain a possible confounding factor, this approach offers a promising step toward objective, biology-based tools for early identification of internalizing symptoms.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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Title: Multimodal prediction of persistent distressing psychotic-like experiences in adolescence using brain imaging, genetic, familial, and environmental markers

Authors: *A. LAL[1], N. KARCHER[4], D. M. BARCH[5], J. LINKERSDOERFER[6], D. J. HAGLER, Jr.[7], J. AHERN[1], D. PECHEVA[6], P. PAREKH[6], T. L. JERNIGAN[2], A. M. DALE[3], C. MAKOWSKI[8];
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Washington Univ., Saint Louis, MO; [7]Multimodal Imaging and Genet., [6]J. Craig Venter Inst., San Diego, CA; [8]Psychiatry, Univ. of California San Diego, San Diego, CA

Abstract: Understanding the neurobiological correlates of psychotic-like experiences (PLEs; e.g., unusual thoughts and perceptual distortions) in adolescence may help identify early biomarkers of psychopathology risk. Utilizing data from the Adolescent Brain Cognitive Development (ABCD) Study, we examined the predictive accuracy of longitudinal brain imaging measures on persistent and distressing PLEs (dpPLEs) in adolescence, alongside genetic, familial, and environmental risk factors. We included 269 individuals with dpPLEs and 6,566 without (ages 8-18 years; baseline: 6,602; 2-year follow-up [2Y]: 4,924; 4Y: 3,875; 6Y: 2,728). Predictors included Restriction Spectrum Imaging (RSI) and Structural MRI (SMRI) vertex-wise cortical measures, including cortical thickness, area, sulcal depth, T1 signal intensities (grey and white matter), and cortical microstructure across restricted, hindered, and free water tissue compartments. Base covariates included age, sex, and scanner. Additional features included genetic polygenic risk scores (PRS) and family history of psychotic disorders, and a summary environmental risk score (e.g. adverse life events). Predictive models using Support Vector Machines (SVM) and Logistic Regression (LogReg) were evaluated across 100 iterations with use of a Stratified fold technique and undersampling to balance the data with robust modelling. Optimal performance for SMRI was achieved with cortical grey-white matter contrast using SVM (AUC=0.69 ± 0.04) and LogReg (AUC=0.70 ± 0.04) when combined with genetic, family, and environmental risk factors (compare to AUC=0.66 ± 0.03 for tissue contrast alone). For RSI, cortical restricted directional diffusion yielded similar results across both methods(AUC=0.70 ± 0.03) when combined with genetic, family, and environmental risk factors. Neuroimaging features capturing putative intracortical myelin show promising predictive utility for adolescent dpPLEs. Prediction is further enhanced by combining genetic, family and environmental risk factors, highlighting the contribution of multiple complex factors to adolescent PLEs, and encouraging further investigation of neurobiological risk factors to inform early intervention strategies in at-risk youth.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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Topic: A.08. Adolescent Development

Support: NIMH Grant 1R01MH120314

Title: Socioeconomic status in early childhood influences neural responses to social evaluation in adolescents

Authors: *C. MCDOWELL, M. A. SHERIDAN, C. C. MERRITT, M. M. DAVIS; Psychology & Neurosci., The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Socioeconomic status (SES) mismatch, when an individual's socioeconomic background is incongruent with the social, cultural, or economic norms of their surrounding environment, may correlate with heightened neural and behavioral responses to social rejection in brain regions known to respond when processing heightened emotional information or during stress induction, including the amygdala, prefrontal cortex (PFC), and hippocampus. Here we take the first step to investigating these phenomena by examining long-term associations between early childhood SES and neural or behavioral responses to social evaluation in late adolescence/early adulthood. In late adolescence/early adulthood, when participants were between 14 and 22 years of age, they completed a social rejection task, the Social Network Aggression Task (SNAT), during fMRI. The SNAT simulates peer rejection, acceptance, or indifference and measures retaliatory behavior (a button press to administer an imaginal noise blast to peer evaluators). We examined predictors of the duration of the imaginal noise blast administered by participants using linear regression models in SPSS. Imaging data was preprocessed and analyzed using FMRIB Software Library (FSL). We examined the association between baseline poverty and noise blast duration by task condition, controlling for biological sex, age, and baseline neighborhood resources (n = 171). We observed that as baseline family income decreased, retaliatory responses to both neutral and positive peer feedback conditions increased, indicating more negative responses for participants from lower SES families. Neural correlates of this association were examined in n = 49 participants. We observed that as family income decreased, activation in the right fusiform region increased for positive feedback trials. This data suggests that early poverty has a long-term impact on participants' experiences of social evaluation, with findings offering implications for resilience and intervention in marginalized youth.

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Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

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BCCH Research Institute BB&D Catalyst Grant

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UBC Faculty of Arts Research Award

Title: Timing of movement and brain dynamics during visuomotor adaptation in children and adults

Authors: *A. J. COOK[1], M. SONG[1], D. GIASCHI[2], H. IM[1];
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Abstract: Visuomotor adaptation refers to the integration of visual and motor processes over time in dynamic environments, supporting motor skill learning through continuous updates to internal models based on perceived movement errors. It develops in childhood but continues to mature, possibly into adulthood. Behavioural studies show that school-aged children can adapt to visual perturbations, although their movements are slower than those of adults. However, the neural mechanisms underlying visuomotor adaptation in children are not well understood. We investigated this by examining the precise timing of brain processes with magnetoencephalography (MEG) in 13 children (6-12 years) and 19 adults (19-40 years) while they performed a child-friendly visuomotor adaptation task. Participants moved a cursor to a target using a computer mouse. During adaptation trials, the cursor was rotated 45° clockwise, requiring hand angle adjustments to reach the target on time. The adaptation period was divided into two stages: early (first half) and late (second half). Both groups adapted by adjusting their hand angles, but children took longer to reach the target than adults in both stages. MEG signals were segmented into epochs that were time-locked to movement onset and were averaged across trials for the early and late adaptation stages respectively. MEG epochs were mapped onto a 3D model of each participant's structural MRI image for source localization of neural activity. Peak latencies of source-localized activations were extracted from visual, parietal, motor, and frontal brain regions. An age effect was observed in the parietal region: during early adaptation, adults showed earlier peak latencies compared to children, with no differences in late adaptation. This finding highlights the parietal cortex as a key region of development in visuomotor control, which is supported by a correlation between behavioural movement time and MEG peak latency (both longer in children) in the parietal cortex during early adaptation. Functional connectivity was estimated using the phase lag index in the alpha frequency range (8-12 Hz), associated with sensory and attentional processing. Children showed stronger frontoparietal connectivity than adults during early adaptation, indicating greater effort, while adults showed stronger connections than children between regions of the default mode network during late adaptation, suggesting the task became less demanding for adults. This research provides new information about the neural dynamics of visuomotor function in children that will also provide a baseline for future studies on visuomotor deficits in developmental disorders.

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Poster

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Program #/Poster #: PSTR372.16/B31

Topic: A.08. Adolescent Development

Title: Inflammatory Gene Expression Impacts Brain White Matter Microstructure in Early Adolescence

Authors: S. AMIRTHA GANESH, J. DODSON, K. NATAN, *L. M. HERNANDEZ;
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Abstract: Emerging research suggests that immune gene expression impacts brain development. In animal models, overexpression of neural-immune genes has been linked to increased microglial engulfment of synaptic processes and reductions in cortical synapse density (Yilmaz et al., 2021). These findings suggest that altered neural-immune gene expression may disrupt age-related synaptic pruning. However, few studies have linked genetically predicted immune gene expression to brain microstructure during development. To address this gap, we examined the impact of genetically regulated expression (GREx) of four key neural-immune genes - *IL6R*, *BDNF*, *NFKBIA*, and *NFKB1* - on magnetic resonance imaging (MRI) measures of brain white matter microstructure and cortical morphology in youth from the Adolescent Brain Cognitive Development (ABCD) cohort. Structural MRI (sMRI; CT, SA, VOL), Diffusion Tensor Imaging (DTI; FA, MD, AD, RD), Restriction Spectrum Imaging (RSI; RNI, RND, RNT) and genetic data were obtained from over 8,500 9/10 year old youth who participated in the ABCD Study. GREx was predicted for the neural-immune genes in youth of European, African and Latinx ancestry leveraging weights from PsychENCODE using TWAS FUSION (Gusev et al., 2016). Linear mixed-effects regressions were performed to test the association between GREx and brain imaging measures controlling for sex, age, socio-economic status, MRI device, family ID and four genetic principal components. Multiple-testing correction was performed using the false discovery rate ($q < 0.05$). Across all four neural-immune genes tested, the most robust associations were found between *NFKBIA* GREx and brain white matter microstructure. Increased *NFKBIA* GREx was associated with higher AD, RD, and MD across regions including white-matter tracts, commissural fibers, projection tracts, subcortical limbic structures, and cortical regions. Conversely, *NFKBIA* GREx was associated with lower RNI in many of the same white-matter tracts, frontal, sensorimotor, and temporal-limbic cortices. Similar effects were observed in subcortical and limbic structures. Higher *IL6R* GREx was associated with increased RNI in the posterior cingulate; no significant associations were detected for *BDNF* or *NFKB1* after multiple testing correction. In summary, genetically predicted *NFKBIA* expression was linked to DTI and RSI measures indicative of reduced white matter microstructural integrity. These findings suggest that elevated *NFKBIA* - despite its inhibitory role in NF-κB-mediated inflammation - may relate to atypical white matter development during early adolescence.

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Poster

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Title: Delayed Maturation of White Matter Tracts during Preadolescence Links to Lower Cognitive Performance and Higher Transdiagnostic Psychopathology

Authors: *D. WANG[1], B. SALMERON[2], X. XIAO[2], A. QUAM[2], J. HILL[2], H. NGUYEN[2], H. LU[2], A. JANES[2], T. J. ROSS[2], Y. YANG[2];
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Abstract: Adolescence is a critical period for the emergence of psychiatric disorders and coincides with rapid white matter (WM) maturation. However, it remains unclear whether deviations from normative WM development contribute to cognitive deficits or psychopathology. In this study, we developed a WM brain age model using tract-wise diffusion MRI features from 10,227 individuals (4,901 females; age 5-21 years) across three large-scale developmental cohorts. The model robustly predicted chronological age in both cross-validation ($R^2 = 0.70$, MAE = 1.805 years) and independent testing. For each participant, the difference between the predicted age and chronological age is defined as WM brain age gap (BAG), which reflects whether the WM development appears less or more mature than expected relative to his/her chronological age. Cognitive performance was evaluated via the NIH Toolbox cognition, and the psychiatric diagnoses were assessed using parent-reported Kiddie Schedule for Affective Disorders and Schizophrenia for DSM-5 (KSADS-5). We found that greater WM BAG (i.e. more advanced WM maturation) was significantly correlated with better fluid ($R = 0.115$, $p < 0.001$), crystallized ($R = 0.109$, $p < 0.001$) and total ($R = 0.130$, $p < 0.001$) intelligence scores, controlling for age and sex. In addition, delayed WM maturation was linked to a greater cumulative number of concurrent ($F = 7.05$, $p_{FDR} = 0.004$) and 2-year-follow-up ($F = 7.03$, $p_{FDR} = 0.005$) KSADS-5 diagnosis. By identifying delay in WM development as a biomarker for cognitive performance and disorder onset, this study provides a valuable framework for tracking brain maturation and understanding the neurobiological underpinnings of cognitive development and transdiagnostic psychopathology.

Disclosures: D. Wang: None. **B. Salmeron:** None. **X. Xiao:** None. **A. Quam:** None. **J. Hill:** None. **H. Nguyen:** None. **H. Lu:** None. **A. Janes:** None. **T.J. Ross:** None. **Y. Yang:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.18/B33

Topic: A.08. Adolescent Development

Support: Brain and Behavior Research Foundation (Young Investigator Grant) ED

Title: Structural Disadvantage - Shared Neural Signatures of Socioeconomic Status, Scarcity, and Neighborhood Threat in Youth

Authors: *K. BROSCH[1], E. DHAMALA[2];
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Abstract: Introduction:
Early life adversity includes environmental, relational, and biological stressors occurring during critical periods of development and is a known risk factor for psychopathology. Specific adversity types, such as neighborhood threat or abuse, have been linked to distinct neural alterations, yet the relative and cumulative effects of different exposures on neurodevelopment remain unclear. Prior studies often focus on single exposures or use univariate methods that may miss shared variance. Here, we used a multivariate, brain-based approach to examine how diverse adversity exposures relate to cortical thickness in youth, aiming to identify both distinct and overlapping neurobiological signatures across development.**Methods:**
We analyzed data from the Adolescent Brain Cognitive Development (ABCD) Study at baseline (N = 6,908; ages 9-10), two-year (N = 5,808; ages 11-12), and four-year follow-up (N = 2,245; ages 13-14). Cross-validated ridge regression models were used to assess associations between regional cortical thickness (Desikan-Killiany atlas) and six adversity dimensions identified by Orendain et al. (2023): physical and sexual abuse, neighborhood threat, scarcity, household dysfunction, prenatal substance exposure, and parental psychopathology. We also included socioeconomic status, given its established links to brain structure and its potential to amplify or confound adversity effects.**Results:**
Cortical thickness was significantly associated with neighborhood threat and socioeconomic status at all time points, and with scarcity at baseline. These associations were strongest in medial temporal and occipital regions and consistent across time, converging on areas involved in memory, visual processing, and higher-order cognition. No significant associations were found for abuse, prenatal substance exposure, household dysfunction, or parental psychopathology. Boys and girls showed similar patterns, with minimal sex-specific effects.**Conclusions:**
Neighborhood threat, scarcity, and socioeconomic status were reliably linked to cortical thickness, showing convergent neural patterns across development despite weak behavioral correlations. These findings suggest a shared neurobiological signature of socioeconomic disadvantage. Our results underscore the potential for environmental adversity to exert widespread, cumulative influence on adolescent brain development, reinforcing the importance of addressing socioeconomic inequality as a key factor in promoting neurodevelopmental health.

Disclosures: K. Brosch: None. **E. Dhamala:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.19/B34

Topic: A.08. Adolescent Development

Support: NSF Grant 2207733
NSF Grant 2116707

Title: Social Jetlag has Detrimental Effects on Adolescent Brain Structures, Network Organization and Intrinsic Dynamics

Authors: M. RISNER[1], ***C. STAMOULIS**[2,1];
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Abstract: This study investigated associations between social jetlag and developing brain circuits and structures in adolescents. N = 3507 youth (median (IQR) age = 12.0 (1.1) years; 50.9% females) from the Adolescent Brain Cognitive Development (ABCD) cohort were studied. Social jetlag (adjusted for sleep debt (SJL_{SC}) versus non-adjusted (SJL)), topological properties and intrinsic dynamics of resting-state networks, and morphometric characteristics were analyzed. Over 35% of participants had SJL_{SC} ≥2.0 h. Boys, Hispanic and Black non-Hispanic youth, and/or those at later pubertal stages had longer SJL_{SC} (β=0.06 to 0.68, CI=[0.02, 0.83], $p \leq 0.02$), which was also associated with higher BMI (β=0.13, CI=[0.08, 0.18], $p<0.01$). SJL_{SC} and SJL were associated with weaker connections between the thalamus and the rest of the brain (β=-0.22, CI=[-0.39, -0.05], p=0.03), potentially reflecting a disrupted sleep-wake cycle. Longer SJL_{SC} was also associated with less topologically resilient and weakly connected salience network (β=-0.04, CI=[-0.08, -0.01], p=0.04), and lower thickness and/or volume of cortical and subcortical structures overlapping with this and other networks supporting emotional and reward processing and regulation, as well as social function (β=-0.08 to -0.05, CI=[-0.12, -0.01], $p<0.05$). SJL_{SC} and SJL were associated with alterations in spontaneous brain activity and coordination that indicate disrupted neural maturation and plasticity. SJL was associated with lower information transfer between regions supporting sensorimotor integration, social function and emotion regulation (β=-0.07 to-0.05, CI=[-0.12, -0.01], $p<0.04$). These findings suggest that misaligned sleep may have detrimental effects on adolescent brain circuit organization and dynamics, and structural characteristics of regions that play critical roles in cognitive function and regulation of fundamental biological processes.

Disclosures: M. Risner: None. **C. Stamoulis:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.20/B35

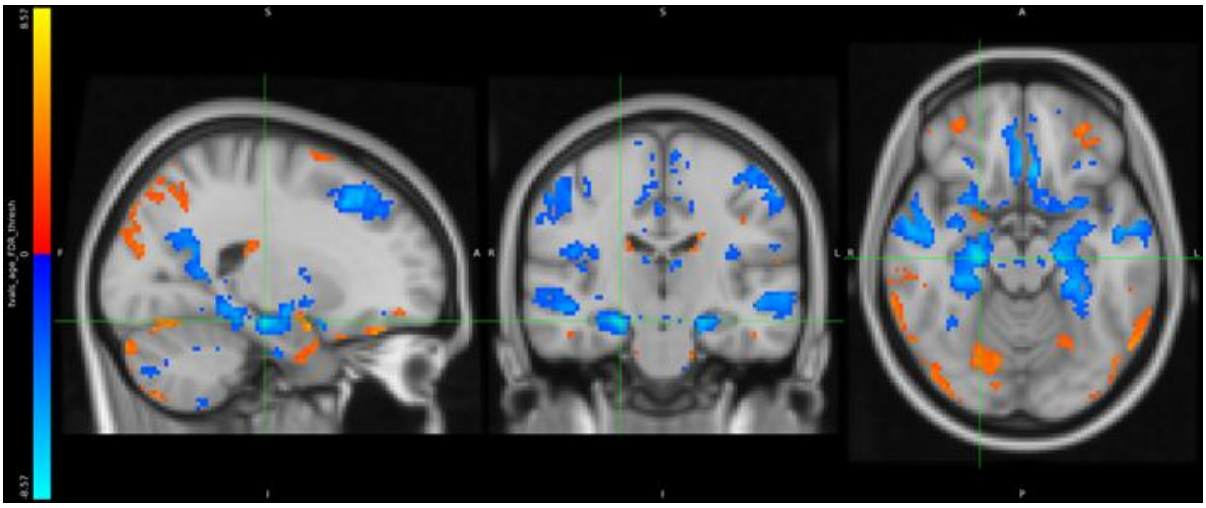
Topic: A.08. Adolescent Development

Support: NIMH Intramural Research Program project ZIAMH002781

Title: Adolescent subgenual anterior cingulate cortex developmental trajectory in ABCD

Authors: ***J. H. LENTZ**[1], E. GIAMPETRUZZI[1], D. M. NIELSON[2], D. S. PINE[1], F. PEREIRA[2], A. ZUGMAN[1];
[1]Emotion and Develop. Br., [2]Machine Learning Team, NIH, Bethesda, MD

Abstract: The subgenual Anterior Cingulate Cortex's (sgACC) connectivity with the dorsolateral Prefrontal Cortex (dlPFC) has been linked to treatment response for depression in adults, both in antidepressant and transcranial magnetic stimulation (TMS) treatment. However, in adolescents there is less evidence that this connection is a factor, or whether the strength of the connection changes with age. The investigation of this potential biomarker is complicated by the fact that the sgACC region is particularly susceptible to signal dropout, causing a low temporal signal to noise ratio (tSNR). It is not known how this connection changes over time and how preprocessing of functional Magnetic Resonance Imaging (fMRI) data affects the results. We hypothesized that the sgACC's seed-based functional connection to other regions would show changes throughout development in adolescence. In this work we use data from healthy human youth in the Adolescent Brain Cognitive Development (ABCD) study (n = 1051, 51% female, mean age = 10.93, age range 8-15, *n scans* = 1617). We ran the following mixed effects model, where y is the seed-based connectivity of the sgACC for each scan: y ~ Age*Sex + 1 + (1|MRI Manufacturer/Collection Site) + (1|Family in ABCD). We found that sgACC connectivity varied with age in our sample, as is shown in **Figure 1**; colored voxels show a significant change in connection with the sgACC by age after false discovery rate correction for multiple comparisons. Blue voxels became less functionally connected to the sgACC with age, and red became more connected. However, these associations appear to be mostly driven by tSNR of the sgACC, as when it is included in the model the effects largely disappear. Future work should take this signal issue into consideration, especially when developing fMRI based targeting approaches for depression in this age range. The results shown here have implications for current seedmap-based interpolation methods used in such TMS targeting research, as age specific seedmaps may be necessary.



Disclosures: J.H. Lentz: None. **E. Giampetruzzi:** None. **D.M. Nielson:** None. **D.S. Pine:** None. **F. Pereira:** None. **A. Zugman:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.21/B36

Topic: A.08. Adolescent Development

Support: NIMH Intramural Research Program project ZIAMH002781

Title: Anxiogenic movie viewing reveal changes in large-scale brain networks in youth with and without anxiety

Authors: *E. CHEN[1], P. QAMAR[2], J. LENTZ[2], P. KIRK[2], A. ZUGMAN[2], D. S. PINE[2]; [1]Emotion and Develop. Br., Natl. Inst. of Hlth., Rockville, MD; [2]Emotion and Develop. Br., Natl. Inst. of Hlth., Bethesda, MD

Abstract: Neuroimaging studies have linked the salience network (SN) and default mode network (DMN) to mood disorders, yet few have examined how these networks respond to naturalistic stimuli such as anxiogenic movies. This study addresses that gap by comparing SN and DMN connectivity in pediatric patients with anxiety and healthy controls during rest and movie viewing. Participants were children and adolescents (ages 8-17) with a primary anxiety diagnosis (n = 31) and age-matched healthy controls (n = 39). Each completed a functional MRI scan with three runs: a resting-state baseline, an anxiogenic movie, and a post-movie resting-state. We hypothesized that (1) participants would show increased SN and decreased DMN connectivity during movie viewing relative to rest, and (2) anxious participants would exhibit greater changes in response to the movie, reflecting heightened sensitivity to affective

cues.Mixed ANOVAs revealed a significant main effect of Condition (pre-movie rest, movie watching, and post-movie rest) on connectivity patterns (ps < .001). Within SN, within DMN, and between SN-DMN connectivity was significantly higher during movie-watching compared to pre- and post-movie rest (*p*s < .03). We did not detect any Condition x Diagnosis interactions (Anxiety or Healthy, *ps* > .742), suggesting that connectivity changes were consistent across groups.Contrary to our expectations, DMN connectivity increased during movie-watching, despite the externally focused nature of the task. One possibility is that the anxiogenic film evoked internally directed processes, such as self-referential thought or autobiographical recall, that can engage the DMN even in perceptual tasks. Notably, this pattern emerged in both anxious and healthy youth, suggesting that DMN and SN engagement during emotionally salient movies may reflect a potentially normative or adaptive mechanism. Further research is needed to clarify whether this response supports or compensates for affective processing demands.

Disclosures: E. Chen: None. **P. Qamar:** None. **J. Lentz:** None. **P. Kirk:** None. **A. Zugman:** None. **D.S. Pine:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.22/Web Only

Topic: A.08. Adolescent Development

Support: R00 MH125021
R01 MH067924

Title: Brain criticality emerges with developmental shifts in excitation-inhibition balance, setting the stage for cognitive control in adulthood

Authors: *A. WESTBROOK[1], F. J. CALABRO[2], A.-E. AVRAMIEA[5], K. LINKENKAER-HANSEN[6], S. MCKEON[3], B. LUNA[4];
[1]Rutgers Univ., Piscataway, NJ; [2]Departments of Psychiatry and Bioengineering, [3]Bioengineering, [4]Dept. of Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [5]Integrative Neurophysiol., VU Univ. Amsterdam, Amsterdam, Netherlands; [6]Vrije Univ., Amsterdam, Netherlands

Abstract: Adult brains, at rest, operate near criticality - at the boundary between super-critical, excitation-dominance, and sub-critical, inhibition-dominance, with qualitatively distinct dynamics on either side. A special set of properties emerge when neural systems operate near criticality, including maximal susceptibility to stimulation, dynamic range, and information transmission, with profound implications for cognitive control. More effective inter-regional signaling, for example, should strengthen top-down control over sensorimotor processing, while greater susceptibility should bias flexibility over the stability of neural representations. Because

proximity to criticality is regulated by the balance of excitation to inhibition, we hypothesize that shifts in inhibitory versus excitatory neurotransmission across adolescent development imply developmental shifts with respect to criticality. We further hypothesize that a systematic shift in brain criticality will affect higher-order cognition. Here, we leverage the dynamical properties of longitudinally collected resting state EEG recordings during N = 310 sessions from 169 healthy human participants ranging in age from 10 to 33 years old. We find that adult brains operate closer to criticality, including broadband increases in long-range temporal correlations and amplitude multistability. Across all ages, individuals whose brains operate closer to criticality during eyes open rest show greater trial-wise variability in accuracy and reaction times during a memory-guided saccade task. The developmental shift towards criticality is mediated by decreasing excitation versus inhibition in the mechanisms driving low-frequency (theta to alpha) oscillations and greater excitation in the mechanisms driving high-frequency (gamma) oscillations. These patterns indicate a developmental shift towards decreasing excitatory versus inhibitory signaling in oscillatory systems associated with top-down control. Finally, we find that higher excitation versus inhibition in alpha, beta, and gamma bands correspond with increased top-down control, reflected in fewer anti-saccade errors and faster responses.

Disclosures: A. Westbrook: None. **F.J. Calabro:** None. **A. Avramiea:** None. **K. Linkenkaer-Hansen:** None. **S. McKeon:** None. **B. Luna:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.23/B37

Topic: A.08. Adolescent Development

Title: Developmental Shift from Reactive to Proactive Inhibitory Control: Longitudinal fMRI Evidence from the ABCD and IMAGEN studies

Authors: *Z. GAO[1], L. ZHENG[5], N. BRANIGAN[2], H. GARAVAN[6], V. MENON[3], W. CAI[4]; [1]Stanford Univ., PALO ALTO, CA; [3]Psychiatry & Behavioral Sci., [2]Stanford Univ., Stanford, CA; [4]Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA; [5]Psychology, Univ. of Arizona, TUCSON, AZ; [6]Psychiatry, Univ. of Vermont, Burlington, VT

Abstract: Inhibitory control, the ability to suppress appropriate responses, undergoes substantial maturation from childhood through adolescence into young adulthood. The dual cognitive control model proposes that inhibitory function comprises both reactive (response cancellation) and proactive (anticipatory preparation) control mechanisms. While behavioral evidence suggests children increasingly transition from reactive to proactive strategies with age, the neural underpinnings underlying this developmental shift remain poorly understood. This study investigates the neurodevelopmental trajectory of inhibitory control mechanisms using comprehensive longitudinal task fMRI data from two major cohorts: the Adolescent Brain

Cognitive Development (ABCD) study (Year 0: ages 9-10, Year 2: ages 11-12; n=2,252) and the IMAGEN study (ages 14, 16, and 19; n=1,061). Participants completed a stop-signal task while undergoing fMRI. We employed an innovative analytical approach combining single-trial modeling with representational similarity analysis to quantify the spatial stability of neural activation patterns. Reactive control was operationalized as the difference in spatial stability between Successful Stop (SS) and Go trials, while proactive control was indexed by the similarity in activation patterns between these trial types. Both cohorts demonstrated significant longitudinal improvements in inhibitory performance, including faster stop-signal reaction times (SSRT), reduced response latencies, and enhanced accuracy (all ps<0.001). In the ABCD dataset, RSA revealed that younger participants showed greater reliance on reactive control mechanisms, evidenced by high spatial stability within salience (SN), frontoparietal (FPN), and visual (VIS) networks. However, this reactive pattern significantly diminished with age, paralleled by increased spatial similarity supporting proactive control, particularly within SN and VIS networks. These developmental trends were replicated in the IMAGEN dataset. These convergent findings from two independent longitudinal cohorts provide compelling evidence for a neurodevelopmental shift from reactive to proactive inhibitory control strategies during the transition from childhood to adolescence. This shift likely reflects a broader maturational reorganization of cognitive control networks, offering important insights into the neural mechanisms underlying the development of self-regulation.

Disclosures: Z. Gao: None. **L. Zheng:** None. **N. Branigan:** None. **H. Garavan:** None. **V. Menon:** None. **W. Cai:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.24/B38

Topic: I.10. Human Learning and Cognition

Support: NSTC113－2410－H－003－034－MY3

Title: Association of GABAergic system gene polymorphisms and signaling with cognitive reasoning and scientific inquiry skills in adolescents

Authors: *L.-C. LEE[1], M.-T. SU[2];
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Abstract: GABAergic signaling, the central inhibitory system in the brain, is fundamental for shaping neuronal excitability, synchronizing neural network activity, and maintaining a critical balance with excitatory glutamatergic transmission. This precise modulation by GABA is critically involved in diverse cognitive functions, including learning and memory, attention, and

executive control. To investigate the association between genetic variations in GABAergic signaling components and specific cognitive abilities in adolescents, this study analyzed data from 669 senior high students. Genetic profiles were correlated with measures of cognitive reasoning and scientific inquiry skills, derived from their academic performance. Our findings reveal significant associations between specific single nucleotide polymorphisms (SNPs) in genes encoding GABAergic receptors - specifically GABBR2 (rs10985765), GABRR2 (rs282129), GABRQ (rs3810651), and GABRE (rs1139916) - and students' cognitive reasoning and scientific inquiry skills. Ongoing research is dedicated to elucidating the precise molecular mechanisms by which these identified SNPs influence the function of their respective genes and subsequently modulate overall GABAergic signaling, thereby impacting cognitive abilities. These findings provide preliminary insights into the genetic underpinnings of cognitive skills relevant to academic success.

Disclosures: L. Lee: None. **M. Su:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.25/B39

Topic: I.10. Human Learning and Cognition

Title: Intense cognitive effort and psychogenic non-epileptic seizures in adolescent girls: two case studies from Cameroon

Authors: *R. MBANGMOU;
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Abstract: Introduction Psychogenic non-epileptic seizures are paroxysmal episodes with an epileptic appearance, but without abnormal brain electrical activity. The role of emotional factors in triggering these episodes is well documented, particularly in adolescents. However, few studies highlight the impact of intense cognitive effort, such as academic overload, as a specific trigger. This article presents two clinical cases illustrating this dynamic in a Cameroonian context. Method Two adolescent girls enrolled in the science series (série D) have been monitored for psychogenic crises for several years: Maeva, 20, in her fourth year of high school, had been having several seizures a day for over three years. Despite a normal EEG, she was treated unsuccessfully with antiepileptic drugs. Clinical analysis showed a marked increase in seizures during exam periods. Adjustment of the learning rhythm (shorter sessions and longer breaks) led to a reduction of over 75% in episodes. During school vacations with no intellectual activity, no seizures were reported. Jenifer, aged 17, also in her third year of secondary school, attended intensive rehearsal courses during classes and vacations. She presented a similar clinical profile, with a strong correlation between cognitive overload and seizure frequency.Résultats Both patients showed a marked reduction in seizures following cognitive load management

strategies and psychotherapeutic support focused on performance anxiety. No pharmacological treatment was required after readjustment of the diagnosis. Discussion These observations highlight the importance of cognitive effort as an underestimated precipitating factor in CNEP in adolescent girls, particularly in demanding academic streams. Individualized management that takes account of mental workload at school could prevent relapses and avoid diagnostic errors. Conclusion In addition to classic emotional factors, excessive cognitive effort should be considered as a potential trigger for CNEP. These cases suggest the urgent need for an adapted pedagogical approach and early psychological assessment in the school context.

Disclosures: R. Mbangmou: None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.26/B40

Topic: E.08. Multisensory Integration

Support: Austin Faculty Funds
NARSAD Young Investigator Award
NIH Grant P50 HD105353
NIH Grant T32 NS105602
NIH Grant T32 CA009206
NIH Grant T32 GM140935
NIH Grant HD094715-01A1

Title: Brain stem contributions to sensory features in autistic children

Authors: *A. BLOCK[1,3], N. ADLURU[2,3], J. GUERRERO-GONZALEZ[4,3], G. KIRK[5,3], A. ALEXANDER[4,3], B. TRAVERS[5,3];
[1]UW-Madison, Madison, WI; [2]UW-Madison, Verona, WI; [3]Waisman Ctr., Madison, WI; [4]Univ. of Wisconsin - Madison, Madison, WI; [5]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Intro. Sensory features are a core component of autism, as up to 74% of autistic children have documented differences in the way they respond to sensory stimuli. Emerging evidence suggests that the brain stem may play an important role in the neurological basis of sensory features in autism. And yet, little is known regarding how the brain stem may be contributing to different components of sensory features in autistic youth. Methods. We explored the relationship between sensory feature components and brain stem white matter microstructure in 37 autistic participants aged 6 to 11 years old. Leveraging brain stem optimized processing techniques of diffusion weighted images, we assessed fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), orientation dispersion index (ODI), intra-cellular volume fraction (ICVF), and longitudinal relaxation rate (R1) of 23 bilateral

fiber bundles extracted from a probabilistic brain stem atlas. To assess different components of sensory features, we utilized items from the evaluation of ayres sensory integration (EASI), an observed measure of sensory responses across several different domains (visual, proprioceptive, postural, etc.). We applied fdr correction and a p value of <.05 to determine significant relationships. Results. Among several relationships between brain stem white-matter microstructure and sensory features, our results indicate a particularly significant relationship between R1 and one component of postural control, specifically a child's ability to maintain a supine flexion position. These relationships were observed in the middle cerebellar peduncle and right parieto-occipito-temporo-pontine tract, as well as bilaterally in the medial lemnisci, superior cerebellar peduncles (spinocerebellar tracts), spinothalamic tracts, corticospinal tracts, inferior cerebellar peduncles (vestibulocerebellar & medullocerebellar tracts), and frontopontine tracts. Discussion. As R1 is thought to be reflective of myelination, our results provide evidence that myelination of several ascending tracts and cerebellar output pathways in the brain stem may be related to individual differences in supine flexion in autistic children. The EASI utilizes supine flexion as a measure of supine postural control, as the position requires the child to elevate their head, arms, and legs from the floor while laying on their stomach. Thus, our results suggest that increased myelination in these tracts may contribute to better supine postural control. Additional research is required to determine how this relationship differs in autistic versus non-autistic children.

Disclosures: A. Block: None. **N. Adluru:** None. **J. Guerrero-Gonzalez:** None. **G. Kirk:** None. **A. Alexander:** None. **B. Travers:** None.

Poster

PSTR372: Adolescent Brain Development, Psychopathology, and Environmental Influences

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR372.27/B41

Topic: A.07. Development of Neural Systems

Support: ZIA MH002928

Title: Multimodal Characterization of Adolescence Neurodevelopment in Non-Human Primate

Authors: *M. ANDUJAR[1], H. E. YARUR[3], S. SHIRLEY[5], S. R. WHITE[6], J. SHIN[7], H. C. GOLDBACH[5], H. A. TEJEDA[4], V. A. ALVAREZ[2], B. B. AVERBECK[8];
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Abstract: Adolescence is a crucial developmental stage marked by profound transformations in brain functions and behavior. These changes are particularly pronounced in the prefrontal cortex

(PFC), where protracted anatomical maturation and synaptic remodeling coincide with the emergence of higher-order cognitive abilities. Despite extensive research on these processes individually, an integrated understanding of how behavioral changes, neural computations, and structural plasticity co-evolve within the same individual remains limited. Recent theoretical models suggest that the pruning of excitatory synapses during adolescence may optimize neural computations by enhancing signal stability and improving task performance, particularly in working memory and reinforcement learning. To evaluate these predictions, we conducted a multi-modal investigation of adolescence development in non-human primates, integrating reinforcement learning-based behavioral modeling, in vivo neuroimaging, and ex vivo cellular and physiological analyses. Behavioral results reveal that adolescent monkeys show reduced learning from positive feedback compared to adults, consistent with less accurate value representations. At the neural level, structural MRI revealed significant group differences in cortical thickness. To uncover the cellular basis of these macrostructural changes, we combined neuroimaging with histological analysis in the same individuals. Quantification of neuronal density and myelin markers revealed that cortical thinning in prefrontal areas was not primarily due to neuron loss or somatic shrinkage, but rather to increased intracortical myelination. While increased myelination appears to drive cortical thinning across multiple frontal areas, region-specific age effects were observed in measures of excitatory/inhibitory (E/I) balance and synaptic pruning. Specifically, the dorsolateral prefrontal cortex (area 46) showed pronounced developmental changes in both spine density and physiological markers of synaptic transmission, indicative of an ongoing reorganization of local microcircuits during adolescence. In contrast, ventromedial prefrontal cortex and striatal regions did not exhibit comparable age-related shifts, underscoring the selective and heterogeneous nature of cortical maturation. By combining behavioral modeling, neuroimaging, and histological analyses in the same individuals, our study helps clarify how different cellular mechanisms may contribute to developmental changes observed at the macrostructural and functional levels.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.01/B42

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

Support: NSERC Discovery Grant 313915

Title: Expression and localization of NMDA receptor GluN2 subunits in dorsal horn pain circuits across sex, species, and late postnatal development

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Abstract: Despite being essential mediators of pain processing, the molecular identity of N-methyl-D-aspartate receptor (NMDAR) subtypes in nociceptive dorsal horn circuits is poorly understood, especially between sexes and in humans. Given the importance of GluN2 subunits in shaping NMDAR function and plasticity, we investigated the expression and localization of specific GluN2 NMDAR variants in the dorsal horn of viable spinal cord tissue from male and female rodents and human organ donors. Analysis of single-cell/nuclei sequencing datasets revealed that the GluN2A (*GRIN2A*) and GluN2B (*GRIN2B*) subunits are robustly expressed in dorsal horn neurons of both mice and humans, with moderate expression of GluN2D (*GRIN2D*) and minimal expression of GluN2C (*GRIN2C*). Immunohistochemistry with antigen retrieval demonstrated that GluN2A, GluN2B, and GluN2D proteins are all preferentially localized to the superficial dorsal horn of both adult rats and humans, which is conserved between males and females. Surprisingly, we found that these GluN2 NMDAR subunits are enriched in the lateral superficial dorsal horn for rats but not for humans, while presynaptic and neuronal markers are symmetrically distributed across the rat mediolateral axis. A dramatic shift in localization of GluN2A to the lateral superficial dorsal horn was observed across later postnatal development (PD21-PD90) in both male and female rats, with a corresponding change in synaptic NMDAR currents. This discovery of changes in NMDAR subunit distribution into rodent adulthood and between species will shed light on the physiological roles of NMDARs and their utility as potential therapeutic targets for pain.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.02/B43

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

Support: the World Premier International Research Center Initiative (WPI), MEXT, Japan
JSPS Bilateral Program Number JP120247404

Title: Single-molecule nanodynamics of AMPARs with non-competitive inhibitors by high-speed atomic force microscopy

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Abstract: Structural biology reveals that the three-dimensional atomic structures of proteins, providing insights into their molecular mechanisms. Moreover, visualizing protein flexibility can deepen our understanding of proteins function. High-speed atomic force microscopy (HS-AFM) is a powerful technique for directly visualizing the flexible regions of single molecules under near-physiological conditions, with a temporal resolution of approximately 100 millisecond at a nanometer scale resolution (*1-3*). α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptors (AMPARs) are essential for rapid excitatory synaptic transmission by localizing to the postsynaptic density of glutamatergic spines. AMPAs are pivotal in drug discovery for epilepsy due to their central role in neuroexcitation. Recent studies have demonstrated that non-competitive inhibitors of AMPARs, such as perampanel (PER), are effective therapeutic agents for epilepsy. AMPARs possess large extracellular N-terminal domains (NTDs) that participate in synaptic clustering. However, the dynamics of these NTDs and the molecular mechanism governing their synaptic clustering, as well as the nano-dynamics of AMPARs upon inhibition by PER, remain unclear. 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, both with and without PER. HS-AFM videos of GluA2-TARP γ2 in 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 (4). Additionally, our findings indicate that the dimeric structure of the flexible NTDs frequently splits into monomers upon binding with PER. Consequently, our findings illuminate the significance of NTD dynamics in the synaptic clustering of AMPARs and inhibition mechanisms by non-competitive inhibitors.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" *ACS Nano* 18, 25018-25035 (2024).

Disclosures: K. Sato: None. **Y. Zhao:** None. **M. Hattori:** None. **M. Shibata:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

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Program #/Poster #: PSTR373.03/B44

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

Support: R01AG085666
R21AG072905
Ellison foundation

Title: Genotype- and region-specific changes in the presence of atypical astrocytes following mild traumatic brain injury in mice

Authors: *J. B. BLACKMAN[1], R. G. KRAUSS[2], S. QUINONES[3], P. TIRJA[4], M. E. SOMMER[1], S. ROBEL[5], T. R. ANDERSON[6], C. G. DULLA[7];
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Abstract: Mild traumatic brain injury (mTBI) affects about 42 million people annually and is a risk factor for Alzheimer's Disease (AD). However, the cellular and molecular brain changes following mTBI and how they contribute to disease pathophysiology remain poorly understood. After the initial injury, disruptions to biochemical processes can induce secondary injury, which can lead to excitotoxicity and neuroinflammation. Some of these processes are mediated by astrocytes, which play key homeostatic roles such as regulating neurotransmitters, extracellular ions, and inflammation. Astrocytes express the Excitatory Amino Acid Transporters GLAST and GLT-1, which help prevent excitotoxicity by regulating glutamate levels. Recent findings have shown an atypical astrocyte (AtA) response following mTBI, marked by substantial loss of key astrocyte proteins (e.g. GLAST, GLT-1, Kir4.1) in areas with no upregulation of inflammatory markers (GFAP). This novel astrocyte subtype has been previously studied using a modified Marmarou repeated-mTBI model in WT mice. Here, we invoke a single injury through a weight drop with rotational-acceleration forces to examine AtAs in both WT (C57BL/6) and a knock-in mouse model of AD (APP^{NL-G-F}). Mixed-sex adult mice were subjected to a closed-head mTBI (chmTBI), then sacrificed three days or one month later. Using immunohistochemistry, we assessed the percent area of AtAs by quantifying loss of GLAST immunoreactivity in areas where GFAP was not upregulated. We quantified the proportion of AtAs in various cortical areas: regions subjected to the weight's direct impact [anterior cingulate cortex, secondary motor cortex, retrosplenial cortex], and select diffuse brain regions [prefrontal cortex, hippocampus, and the amygdala]. Preliminary findings show that three days following chmTBI, both WT and AD mice demonstrate a significant injury-induced increase in the percent area of AtAs in focal areas and the amygdala. Interestingly, injured AD mice had significantly more AtAs than injured WT mice in focal, but not other cortical regions, indicating a genotype- and region-specific effect of injury. One-month post-injury, both genotypes showed significant decreases in the percent area of AtAs in focal and hippocampal regions compared to three days post-injury. These results suggest that AtAs may be transient, with region-specific recovery of key homeostatic astrocyte proteins regardless of an AD phenotype. Our findings provide insight into astrocytic changes following mTBI, which may aid in the field's understanding of how secondary injury unfolds in relation to neurotransmitter homeostasis and other astrocyte functions.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.04/B45

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

Support: NIH Grant #R01AG069266
WW Smith Foundation

Title: Characterizing molecular and behavior phenotypes in a novel GluN2B knockin model with altered PDZ-binding

Authors: *S. SUBRAMANIAN[1], A. SANZ-CLEMENTE[2];
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Abstract: NMDA Receptors (NMDARs) are ionotropic glutamate receptors involved in key processes, including synaptic plasticity, neuronal network regulation, and cognition. NMDAR-related research has mainly focused on *synaptic* NMDARs (sNMDARs), receptors that are stabilized at post-synaptic densities (PSDs). However, around one-third of functional NMDARs are extrasynaptic (exNMDARs), i.e, receptors located over 100 nm away from the PSD that are not activated by low-frequency stimulation. The physiological functions and regulation of exNMDARs are poorly understood, although *ex vivo* studies have involved them in controlling neuronal excitability and neuronal/glial cross-talking. Additionally, exNMDARs are translationally relevant as they are the major mediators of excitotoxicity. s- and exNMDARs are stable subpopulations, yet they are interconnected through lateral diffusion. It has been hypothesized that the regulated modulation of s/exNMDAR influences synaptic plasticity and higher cognitive functions. However, experimental confirmation of this speculation is lacking. A major obstacle to advancing our knowledge about the significance of s/exNMDAR balance as well as exNMDAR physiological function and regulation is the lack of suitable research tools, including animal models. We have generated novel knock-in (KI) mouse lines to help overcome this limitation. Our animals have been designed based on our previous findings showing that the phosphorylation of the PDZ ligand of the GluN2B subunit of NMDAR is a crucial regulator of receptor localization. Briefly, non-phosphorylated GluN2B anchors NMDARs at the synaptic sites via MAGUK binding. Phosphorylation disrupts this interaction, causing NMDARs to relocate to extrasynaptic membranes. Therefore, we have developed our KIs to express either phospho-deficient or phospho-mimetic mutants of GluN2B in both a conditional (Cre-dependent) and a constitutive manner. Heterozygous and homozygous from both phospho-deficient and phospho-mimetic lines were viable and observed at expected Mendelian ratios. Initial biochemical and behavioral characterization indicates the anticipated alteration in sNMDAR content and suggests locomotor alterations. We are currently conducting an in-depth characterization of our mouse lines, including functional and anatomical analyses of NMDAR subsynaptic location, effects on synaptic and cellular morphology, and behavioral consequences.

If, as expected, our animals exhibit an altered s/exNMDAR balance, they will facilitate the *in vivo* study of exNMDAR determinants, NMDAR-plasticity, and their role in excitotoxicity and associated neuropathies.

Disclosures: S. Subramanian: None. **A. Sanz-Clemente:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

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

Support: Cariplo Foundation (2021–1516) to FG
MIUR-PRIN (Bando 2022, Prot. 2022YY85P5) to FG

Title: At the synapse and beyond: GluA3 in synaptic activity, signalling, and neuronal architecture

Authors: *M. ITALIA, A. SPINOLA, S. CARUSO, E. ZIANNI, J. GOMEZ BLANCO, S. PELUCCHI, E. MARCELLO, M. DILUCA, F. GARDONI;
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Abstract: AMPA-type glutamate receptors (AMPARs) are tetrameric cation-permeable channels composed of GluA1-GluA4 subunits that mediate fast excitatory neurotransmission. Among them, GluA3, encoded by the GRIA3 gene, has been associated with neurodevelopmental and psychiatric disorders. However, *its specific contribution to glutamatergic synapses and downstream signalling pathways remains poorly understood.*
To investigate this, we used shRNA-mediated GluA3 knockdown in primary hippocampal neurons at *day-in-vitro* 10 (DIV10). We initially focused on the postsynaptic compartment, where GluA3 silencing led to altered dendritic spine morphology and reduced expression of the NMDAR2B subunit at synaptic sites. Functionally, spontaneous calcium transients were diminished—independently of presynaptic glutamate release—and single-spine stimulation induced exaggerated potentiation, pointing to impaired synaptic homeostasis.
To probe the downstream impact of this synaptic alteration, we analysed transcriptional profiles. RNA-seq revealed dysregulation of key activity-dependent genes, including CAMK2A and MAPK3, both central to calcium signalling and synaptic plasticity. These results highlight GluA3 as a key regulator of intracellular signalling downstream of synaptic activity.
In parallel, we observed a striking reduction in dendritic arbor complexity, suggesting that synaptic dysfunction may reverberate onto neuronal structure. Interestingly, these effects were highly time-dependent: silencing at DIV3 produced more pronounced phenotypes than at DIV10, while knockdown at DIV14 had minimal impact, indicating a critical developmental window for GluA3 function. Notably, *in vivo* silencing of GRIA3 in the dorsal CA1 region of the

hippocampus in young adult male mice recapitulated the dendritic arbor structural defects observed *in vitro*.
Together, our findings uncover a temporally precise role for GluA3 in shaping synaptic activity, intracellular signalling, and neuronal architecture—*at the synapse and beyond*. Given the implication of GRIA3 variants in neurodevelopmental and psychiatric disorders, this work provides new insights into GluA3-associated pathologies and may inform targeted therapeutic strategies.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.06/B47

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

Support: AIIMS MD Thesis Financial Support-2023
ICMR MD Thesis Grant

Title: Role Of Altered Subunit Configuration Of Glutamate Receptors Towards Dysfunctional Synaptic Activity In BCNU Model Of Focal Cortical Dysplasia (FCD)

Authors: *A. J KUMAR[1], S. NAMBIAR[1], A. B. DIXIT[4], M. TRIPATHI[2], P. CHANDRA[3], J. BANERJEE[1];
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Abstract: Focal Cortical Dysplasia (FCD), the most common substrate of drug-resistant epilepsy in paediatric population, is a neurodevelopmental disorder characterised by abnormal cortical architecture and hyperexcitability. One of the major hypotheses of drug resistance in FCD is target hypothesis where we focus in this study. Reorganisation of synaptic receptor expression in FCD is a crucial consequence of mTOR pathway dysregulation. Different studies in cortical dysplasia tissues have shown different alterations of glutamate receptor subunits but all of them pointing towards hyperexcitability in cortical dysplasia. It has been reported that subunit alterations of NMDA receptor in FCD can alter its kinetics and altered AMPA receptor subtypes have been shown in dysplastic neurons in cortical tubers. Role of GABA receptor alterations have already been well studied in humans as well as animal models of FCD. These findings motivated us to study alteration of NMDA and AMPA receptor subunits in BCNU-rat model of FCD. BCNU model of FCD was developed in Sprague-Dawley rats and at P30, brain samples from the somatosensory cortex were isolated. We performed mRNA and protein expression of the subunits N2b, GluA1 and GluA4 by qPCR and immunohistochemistry respectively. We proceeded to perform Whole-cell patch clamp to record spontaneous excitatory postsynaptic

currents (EPSCs) from pyramidal neurons in the neocortex of the resected brain slices. Our results show significant increase in mRNA expression of GluA1 subunit (AMPAR) in FCD tissue [FCD (ΔCt: 3.36±0.33, n=6, p=0.03) and Control (ΔCt: 2.66±0.41, n=6) and a significant decrease in that of both L and S isoforms of GluA4 (AMPAR) subunit [L-isoform; FCD (ΔCt: 4.27±1.19, n=6, p=0.005) and Control (ΔCt: 6.69±1.08, n=6); S-isoform; FCD (ΔCt: 4.51±1.25, n=6, p=0.008) and Control (ΔCt: 6.71±1.04, n=6)] while no significant change was found in N2b subunit (NMDAR) expression. Immunohistochemistry (n=6 in each group) corroborated the gene expression studies. We observed an alteration in the frequency and amplitude of spontaneous EPSCs in the neocortex of FCD rats (n=6) than in control rats (n=6). Our findings suggest that alteration in glutamate receptor subunit expression contributes to the altered receptor kinetics which may possibly contribute to altered frequency and amplitude of spontaneous EPSCs. This could be responsible for hyperexcitability and increased seizure susceptibility in FCD. These results provide new insights into the molecular mechanisms underlying FCD and may guide the development of targeted therapies for this debilitating condition.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.07/B48

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

Support: CIHR
Krembil Foundation

Title: Alternative splicing of Grin1 gates NMDA receptor blockade and antidepressant activity of ketamine

Authors: *A. T. HE[1,2], W. ZHANG[2], H. LI[2], Y. TU[2], D. LEE[1,2], Z. JIA[1,2], M. W. SALTER[1,2]; [1]Univ. of Toronto, Toronto, ON, Canada; [2]The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Ketamine has emerged as a rapid-acting and robust antidepressant. However, the mechanism of its antidepressant action remains enigmatic. Ketamine is an open channel blocker of NMDA receptors (NMDARs). These receptors are diverse in structure and function depending on their subunit composition. *Grin1* encoding the GluN1 subunit undergoes alternative splicing to exclude exon 5 (GluN1a) or include this exon (GluN1b), which encodes the N1 cassette located in the N-terminal domain distant from the pore. Here we report that alternative splicing of *Grin1* exon 5 unexpectedly dictates the NMDAR blockade of ketamine and gates its antidepressant activity. In this study, we used mice engineered to exclude *Grin1* exon 5 (GluN1a mice) or include this exon (GluN1b mice). We found that ketamine prevented NMDAR-dependent long-term potentiation (LTP) in the CA1 region of the hippocampus in GluN1a mice,

while ketamine had no effect on LTP in GluN1b mice. Ketamine inhibition of synaptic NMDAR currents in CA1 pyramidal neurons was significantly greater in GluN1a than in GluN1b mice. Furthermore, the voltage-dependent relief of ketamine blockade was significantly slower in GluN1a than in GluN1b neurons such that during bursting activity, ketamine blockade persisted in GluN1a-containing NMDARs, whereas ketamine escaped from GluN1b-containing NMDARs. Lastly, ketamine treatment, via systemic administration or local infusion into the hippocampus, induced an antidepressant effect in GluN1a mice but had no effect in GluN1b mice. We therefore conclude that GluN1a-containing NMDARs selectively mediate the antidepressant effect of ketamine.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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

Support: NSTC-110-2628-B-001-018
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NSTC-112-2628-B-001-003
NSTC-113-2311-B-001-025
R01 NS036715

Title: Pathogenic calcium-permeable AMPAR mutation causes neurodevelopmental disorders rescued by ASO therapy

Authors: C.-M. CHEN[1], Y.-M. HUANG[2], C.-C. CHUNG[2], R. C. JOHNSON[1], F.-Y. HSIAO[2], R. L. HUGANIR[1], ***S.-L. CHIU**[2];
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Abstract: AMPA-type glutamate receptors (AMPARs) are central to fast excitatory synaptic transmission in the brain, mediating neuronal development and higher-order functions. A recurrent *de novo* variant in *GRIA1* (A636T), located within a conserved channel gating motif, has emerged as a significant hotspot mutation in individuals with neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD) and intellectual disability (ID). However, the precise mechanisms by which this mutation contributes to NDDs remain elusive. To investigate the pathogenicity of *GRIA1*-A636T, we generated a *Gria1*-A636T knock-in mouse model. This model recapitulates core behavioral deficits relevant to ASD/ID and reveals a selective vulnerability of the hippocampus, characterized by progressive atrophy, dendritic remodeling, and excitotoxic neuronal death. Mechanistically, we show that the A636T mutation significantly

alters AMPAR gating kinetics, leading to an enhanced excitatory drive and an increase in AMPAR calcium permeability. This aberrant calcium entry, coupled with an impaired critical postnatal downregulation of CP-AMPARs in the hippocampus, prolongs a developmental state of heightened neuronal susceptibility to excitotoxicity. Notably, a single neonatal administration of an allele-specific antisense oligonucleotide (ASO) targeting the mutant *Gria1* transcript fully prevented neuronal loss, suppressed neuroinflammation, and normalized hippocampal neuronal structure. These findings establish *GRIA1*-A636T as a key pathogenic gain-of-function mutation that disrupts fundamental AMPAR function, particularly the calcium permeability, and highlight the significant translational potential of precision RNA-targeted therapies for AMPAR-associated NDDs.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.09/B50

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

Title: Genetically encoded toxins for treating NMDAR-related disease

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Abstract: Excessive activation of NMDA receptors (NMDAR) contributes to neurodegeneration in Alzheimer's disease (AD). Notably, extrasynaptic NMDARs enriched in GluN2B subunits are known to mediate synaptic dysfunction and neuronal loss. However, there are very few pharmacological tools available to selectively target and modulate specific NMDAR subtypes (e.g., GluN2B). Moreover, no existing molecular tools allow for restricted modulation of NMDARs within defined brain regions or subcellular compartments. To address this gap, we engineered a genetically encoded, membrane-tethered toxin derived from the spider toxin PnTx4(5-5) (denoted t-PnTx), for selective inhibition of GluN2B-containing NMDARs. Our findings show that t-PnTx acts as a subunit-selective inhibitor, effectively blocking GluN2B-containing NMDAR currents in *Xenopus* oocytes. In primary hippocampal neurons, AAV-mediated expression of t-PnTx significantly reduced the proportion of active extrasynaptic NMDARs. Importantly, t-PnTx expression protected against NMDA-mediated neuronal death in cultured hippocampal neurons. To investigate its effects *in-vivo*, we injected AAVs to express t-PnTx in the hippocampus of adult mice. Electrophysiological recordings from acute hippocampal slices revealed that t-PnTx expression modulated NMDAR-mediated currents and induction of long-term potentiation, suggesting a functional impact on receptor activity in the intact brain. These results position t-PnTx as a novel, genetically targetable strategy for subtype-specific

NMDAR modulation. Ongoing studies in the 5XFAD mouse model are aimed at evaluating its therapeutic potential to mitigate AD-related synaptic deficits and cognitive decline.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.10/B51

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

Support: Wellcome Trust (223131/Z/21/Z)

Title: Role of alpha-5 GABA A receptors in episodic-like memory and object recognition

Authors: *F. RAWLINGS-MORTIMER[1], M. C. D. BAILEY[1], J. F. DU HOFFMANN[2], Z. KOURTZI[1], J. W. DALLEY[1];
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Abstract: Episodic memory is a memory of 'what' an object is, and 'where' and 'when' it is encountered. Although episodic memory is profoundly impaired in neurological disorders such as Alzheimer's disease, there are presently few therapeutic options available to offset episodic memory loss. This study investigated the role of alpha-5-expressing GABA A receptors in episodic-like memory (ELM) in mice using both positive and negative allosteric modulators. This research was motivated by previous research implicating the alpha-5 GABA-A subunit in hippocampal-dependent learning plasticity. We investigated the effects of the alpha-5 GABA A receptor specific positive allosteric modulator alogabat and the negative allosteric modulator BI-1030 on ELM in mice together with a classic novel object recognition (OR) task. For the ELM task, mice underwent three training sessions. In the first session, mice explored four identical objects in set locations. An hour later a second session was undertaken with another four identical objects. Two of the objects were in the same location as the previous session and two in new locations. Immediately following this second stage, the mice were administered BI-1030 (3mg/kg s.c.), alogabat (10mg/kg i.p.) or vehicle. In the test session four hours later, two of the original and two of the recently explored objects were used, located in either stationary or displaced locations. For the OR task, subjects received the same drugs and doses as above one hour prior to object recognition training. They were then tested 48hrs later. We found that the mice that had received BI-1030 spent significantly more time exploring the old, displaced object in the episodic-like memory task compared to the control group, suggesting that they had retained 'what', 'where' and 'when' memory capacities. In addition, long-term object recognition memory was significantly impaired by alogabat administration, with mice spending less time exploring the novel object compared with controls. These findings reveal significant cognitive enhancement following negative allosteric modulation of the alpha-5 GABA A

receptor. By contrast, positive allosteric modulation impaired long term object recognition memory. This dissociation motivates further research on the therapeutic potential of allosteric modulators of alpha-5 GABA A receptors in human memory disorders.

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Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.11/B52

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

Title: Exploring the role of δ subunit-containing $GABA_A$receptors in hippocampus-dependent memory functions

Authors: *N. MAMISASHVILI, O. LANFORD, E. PINTO, M. BROWN, K. NASRALLAH; Biol. Sci., Fordham Univ., Bronx, NY

Abstract: A central goal in Neuroscience is to elucidate the mechanisms underlying complex brain functions and their dysregulation in neurological disorders. Deficits in memory, particularly in memory discrimination—the ability to distinguish between similar experiences—are common in conditions such as autism spectrum disorder, post-traumatic stress disorder, and panic disorders. Understanding the neural substrates of memory processing thus holds broad translational relevance. GABA, the primary inhibitory neurotransmitter in the adult mammalian brain, controls neural circuit function through both phasic (brief) and tonic (persistent) inhibition. Most studies have focused on phasic inhibition, which is mediated by synaptic GABA receptors. However, tonic GABA currents, mediated by non-synaptic receptors, powerfully control neuronal excitability and information processing. Surprisingly, very little is known about how tonic inhibition in specific brain areas and neurons could contribute to memory and behavior. The hippocampal formation, known for its role in memory, is a promising area for investigation. Within this complex structure, the dentate gyrus (DG) is a key relay station that transfers information from the entorhinal cortex into the hippocampus proper. Non-synaptic δ-containing $GABA_A$ receptors (δ-$GABA_A$Rs) are particularly enriched in dentate granule cells (GCs), principal neurons of the DG. Evidence suggests that tonic inhibitory control of GC, including by δ-$GABA_A$Rs, is essential for proper hippocampal function. In this study, we investigate the functional role of δ-$GABA_A$Rs in DG granule cells using a conditional knockout mouse model with targeted deletion of the δ subunit in adult male and female mice. We assess behavioral outcomes using classical Open Field test, Elevated Plus Maze, and Contextual Fear Conditioning to evaluate anxiety-like behavior and hippocampus-dependent memory. In parallel, we employ electrophysiological recordings in acute hippocampal slices and c-Fos immunolabeling to examine the impact of δ-$GABA_A$Rs deletion on DG circuit function. Our findings aim to fill a critical gap in understanding how tonic inhibition shapes hippocampal processing and behavior.

This work could inform new strategies for treating cognitive and emotional disturbances associated with altered GABAergic signaling.

Disclosures: N. Mamisashvili: None. **O. Lanford:** None. **E. Pinto:** None. **M. Brown:** None. **K. Nasrallah:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.12/B53

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

Support: TUBITAK

Title: Benchmarking Pathogenicity Predictors on Epilepsy-Associated GABA(A) Receptor Missense Variants: AlphaMissense Leads in Accuracy

Authors: B. KARADAG[1], ***A. ARSLAN**[2];
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Abstract: Dysfunction of gamma-aminobutyric acid type A ($GABA_A$) receptors, due to variants in genes encoding key subunits, contributes to genetic epilepsy by disrupting excitation-inhibition balance. Next-generation sequencing (NGS) enables the identification of disease-associated variants, yet interpretation remains challenging as many missense variants are classified as variants of uncertain significance (VUS). The ACMG/AMP guidelines recommend incorporating computational prediction models (PP3/BP4 criteria) for variant interpretation, but the accuracy of these models—particularly for gene-specific contexts—remains uncertain. In this study, we benchmarked 21 pathogenicity prediction models on 381 missense variants in GABRA1, GABRB2, GABRB3, and GABRG2—key genes encoding $GABA_A$ receptor subunits—curated from ClinVar and the Human Gene Mutation Database (HGMD). Models were evaluated using accuracy, precision, sensitivity, specificity, F1 score, and Kappa statistics. The machine learning-based AlphaMissense achieved the highest overall performance (accuracy: 92%, F1 score: 95%), while the deep learning model MetaRNN demonstrated the highest precision (91%) and specificity (99%). The latter also had the highest accuracy (91%) and F1 score (95%) among deep learning models. Polyphen2_HVAR and Polyphen2_HDIV were the most compatible models, according to Kappa statistics. Notably, we observed significant inter-model disagreement, highlighting the need for improved consensus strategies. These results support AlphaMissense as the most effective model for interpreting missense variants in $GABA_A$ receptor genes linked to epilepsy, although its performance also underscores the need for further enhancement. Future directions will involve integrating cellular pathology and phenotype-level predictions to refine interpretation of $GABA_A$ receptor gene variants in the context of epilepsy.

Disclosures: B. Karadag: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); TUBITAK, Turkiye. **A. Arslan:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); TUBITAK, Turkiye.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.14/Web Only

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

Title: GT-002, a novel positive allosteric modulator of the $GABA_A$ receptor ($GABA_A$ PAM): preclinical and clinical characterization

Authors: C. N. RYAN, ***M. R. WITT**;
Gabather AB, Södertälje, Sweden

Abstract: GT-002 is a novel therapeutic candidate developed by Gabather for the treatment of central nervous system (CNS) disorders, with a primary focus on cognitive and affective dysfunctions. As a positive allosteric modulator of $GABA_A$ receptors, GT-002 targets neuropsychiatric and neurodegenerative disorders by enhancing inhibitory neurotransmission. Preclinical studies have demonstrated promising effects on cognition, social interaction, and emotional regulation, with favourable safety and pharmacokinetic profiles.
Electrophysiological assays in heterologous expression systems and native neurons revealed that GT-002 exhibits subtype selectivity, with strong potentiation of α2- and α3-containing $GABA_A$ receptors, subtypes implicated in anxiolytic and cognitive functions. This receptor subtype profile supports the potential for a therapeutic effect with reduced side effects compared to non-selective modulators.
A completed Phase I clinical trial - performed as three separate studies, on each occasion at a different clinical site - confirms that GT-002 is safe and well tolerated in healthy human subjects, showing dose-proportional pharmacokinetics and no serious adverse events. In a translational EEG study, GT-002 significantly modulated spectral power in the theta and beta frequency bands, consistent with enhanced cortical inhibition and target engagement at $GABA_A$ receptors. These biomarker changes mirror electrophysiological signatures observed with clinically active CNS agents.
GT-002 is positioned for further clinical development targeting indications such as schizophrenia, depression, and cognitive impairment and a clinical trial in patients suffering from schizophrenia has been initiated in 2025. Strategic partnering opportunities are being explored to support later-stage trials and commercialisation pathways. Overall, GT-002 represents a promising CNS drug candidate with a novel mechanism, favourable receptor selectivity, and strong translational biomarker support.

Disclosures: C.N. Ryan: A. Employment/Salary (full or part-time): Gabather AB. **M.R. Witt:** A. Employment/Salary (full or part-time): Gabather AB.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.15/B54

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

Support: PAPIIT-UNAM-México No. IN215725

Title: β2 (M286) is a molecular determinant for the positive modulatory effect of β-CCB on α3β2γ1 the oligodendroglial $GABA_A$ receptor

Authors: *B. VARELA[1], E. GARAY[2], A. LIMON[3], R. ARELLANO[2];
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Abstract: The $GABA_A$ receptor has been described as a main binding site for β-carbolines. Originally, they have been considered inverse agonists binding to the classic benzodiazepine (BZD) site in neuronal receptors, promoting a negative effect. It has been shown that some β-carbolines such as methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) also act positively through a binding site shared with loreclezole (LCZ), a selective β2/β3 subunit modulator. This same pocket, also share determinants with others modulators such as propofol and etomidate. The oligodendrocyte precursor cells (OPC), and the oligodendrocytes (OL), the central nervous system myelinating cells, express $GABA_A$ receptors ($GABA_AR$) conformed by α3β2γ1 subunits. It has been shown that, unlike neurons, several β-carbolines present predominantly a positive modulation on the $GABA_AR$ of OL, it is the case of n-butyl-β-carboline-3-carboxylate (β-CCB) and ethyl-β-carboline-3-carboxylate (β-CCE). β-CCB is the most potent and selective β-carboline for this $GABA_A$ receptor, and has no effect on the main neuronal receptor (i.e., α1β2γ2), since both receptors contain the β2 subunit; the selectivity is not yet understood. We have shown that the systemic application of β-CCB in in vivo experiments has a promyelinating effect in different models of demyelination; this potential therapeutic use urges to understand the molecular mechanisms that define its selectivity on the α3β2γ1 receptor. In here, we explore the role of 2 important β2 subunit residues involved in loreclezole and propofol binding: the N265 and M286, respectively. For this, were made point mutations in the β2 subunit, and subunits containing N265S or M286W, in combination with α3 and γ1 subunits were evaluated electrophysiologically by expressing the receptors in Xenopus oocytes. Our data indicated that the LCZ pocket participates in the pharmacological action of β-CCB on this $GABA_AR$ and that M286 plays a central role for this positive effect. Considering that the interface with the α3 subunit is also important in the configuration of the LCZ site (α3-β2), this result suggested that other structural elements of the α3β2γ1 receptor are involved in the clear distinction of β-CCB effects between the neuronal and oligodendroglial receptor.

Disclosures: B. Varela: None. **E. Garay:** None. **A. Limon:** None. **R. Arellano:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.16/B55

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

Support: R35NS116852

Title: Brain extracellular matrix alters local anion concentrations and responses to injury

Authors: *K. P. NORMOYLE[1], V. I. DZHALA[2], K. P. LILLIS[4], K. EGAWA[5], K. J. STALEY[3];
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Abstract: The reversal potential of $GABA_A$ receptors (E_{GABA}) is dependent upon the chloride concentrations on both sides of the neuronal membrane. We recently discovered that the extracellular chloride concentration ($[Cl^-]_o$) is non-uniform and half the chloride in bulk cerebrospinal fluid. We also observed that removal of a portion of the immobile polyanions from the extracellular space, mimicking injury-induced metalloprotease (MMP) activation, results in a shift toward higher local $[Cl^-]_o$ *in vitro*. This supports the idea that mobile chloride ions are displaced by the plentiful anionic immobile elements of the extracellular matrix. We used 2-photon Fluorescence Lifetime Imaging (FLIM) of a custom chloride-sensitive fluorophore constrained to the extracellular space by conjugation with 10 kilodalton dextran and 2-photon photolysis of single neurons to model acute brain injuries, first in organotypic cultures of hippocampal slices and then *in vivo* via mouse cortical window implantation. We found a strong dependence of $[Cl^-]_o$ vs distance from photolytic injury, with $[Cl^-]_o$ increasing to ACSF levels in proximity to photolysed neurons, which we hypothesize is due to mobilization of immobile extracellular anions following MMP release and activation. We then tested whether excess immobile extracellular anions lead to low $[Cl^-]_o$. Using 19mo APP/PS1 mice we measured $[Cl^-]_o$ at sites of polyanionic amyloid (Aβ) aggregates that accumulate in extracellular space in Alzheimer's disease. We observed lower $[Cl^-]_o$ in proximity to Aβ aggregate accumulations identified with near-infrared fluorescent stain CRANAD-3. Taken together these data confirm that displacement of $[Cl^-]_o$ by Donnan forces is an underappreciated mechanism by which $[Cl^-]_o$ may become pathologically high by degradation of extracellular polyanions or pathologically low due to accumulations of polyanions, such as Aβ aggregates. These changes in $[Cl^-]_o$ should also alter the neuronal intracellular chloride ($[Cl^-]_i$) via the activity of the high-velocity equilibrative membrane chloride transporters. In adolescent wildtype mice we have observed increased $[Cl^-]_i$ upon acute injury and subsequent $[Cl^-]_o$ increase, as measured with the ratiometric reporter Super Clomeleon. In conclusion, $[Cl^-]_o$ is partially displaced by carboxylates and sulfates in the extracellular matrix. Pathophysiological removal or deposition of extracellular

immobile anions alters the distribution of chloride in both the extra- and intracellular spaces. These findings have immediate implications for the treatment of cytotoxic edema, seizures, and neurodegenerative disorders including Alzheimer's disease.

Disclosures: K.P. Normoyle: None. **V.I. Dzhala:** None. **K.P. Lillis:** None. **K. Egawa:** None. **K.J. Staley:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.17/B56

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

Title: Investigating proteostasis disruption in GABRA1 frameshift mutations and its implications for epilepsy pathogenesis

Authors: *M. WILLIAMS[1], T. MU[2];
[1]CASE WESTERN RESERVE UNIVERSITY, Cleveland, FL; [2]Case Western Reserve Univ., Cleveland, OH

Abstract: The Gamma-aminobutyric acid type A receptor ($GABA_AR$) is the most common inhibitory neurotransmitter-gated ion channel in the central nervous system. Mutations within the $GABA_AR$ can result in an excitation-inhibition imbalance, leading to epilepsy disorders. Specifically, frameshift mutations in the α_1 subunit encoded by the *GABRA1* gene have been associated with epilepsy disorders such as childhood absence epilepsy and developmental epileptic encephalopathy. Several frameshift mutations are characterized by deletions in the gene, leading to truncated subunits. These mutations result in a premature termination codon (PTC), resulting in significant structural effects in the α_1 subunit of the $GABA_AR$. We hypothesize that these variant α_1 subunits present as severely misfolded proteins with impaired trafficking, disrupting proper $GABA_AR$ inhibitory ion channel function. However, due to lacking functional characterization, there is little known about the disease mechanisms that underlie these mutations. This study investigates the frameshift mutation, K401fs425X, previously associated with treatment-resistant epilepsy. Using human induced pluripotent stem cell (iPSC)-derived cortical neurons, $GABA_AR$ subunit expression, neuronal maturation, cell health, and electrophysiological activity were analyzed using western blotting, immunocytochemistry, quantitative PCR, and micro-electrode array recordings. The K401fs425X mutation resulted in impaired proteostasis and neuronal health, and led to deficits in electrophysiological activity. With limited literature on the pathogenesis of frameshift mutant *GABRA1*-linked diseases, this study fills this gap in knowledge and provides insight into mutant $GABA_AR$ proteostasis. Furthermore, these findings elucidate the molecular and functional consequences of *GABRA1* frameshift mutations utilizing a clinically relevant human cell model.

Disclosures: M. Williams: None. **T. Mu:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.18/B57

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

Support: Deutsche Forschungsgemeinschaft VI586 and SO328
SYNABS FOR3004

Title: Glycine receptor autoantibody binding patterns to CNS tissue relate to symptoms in patients

Authors: A.-L. WIESSLER[1], I. PIRO[2], E. TUZUN[3], C. L. SOMMER[2], *C. VILLMANN[1]; [1]Univ. Hosp. of Wuerzburg, Wuerzburg, Germany; [2]Dept. of Neurol., Univ. of Wuerzburg, Wuerzburg, Germany; [3]EMAR Med. Ctr., Istanbul, Turkey

Abstract: Glycine receptor (GlyR) autoantibodies are associated with the rare autoimmune disease Stiff Person Syndrome (SPS) or the more severe form Progressive Encephalomyelitis with Rigidity and Myoclonus (PERM) as well as with encephalitis or epilepsy. The targeted GlyRs are located at pre- and postsynaptic sites mainly in spinal cord and brainstem. Additionally, GlyR subunits are also expressed in brain areas such as hippocampus, amygdala, thalamus and hypothalamus as well as in cerebellar cell layers, however subunit compositions of GlyRs in spinal cord may differ from higher brain areas. At the molecular level, different GlyRα subunits and the GlyRβ subunit were identified as targets of GlyR autoantibodies. Binding of autoantibodies to GlyRs interferes with inhibitory signal transmission. Patients suffer from a variety of symptoms. Main symptoms in SPS/PERM patients are spasms and stiffness of skeletal muscles. How the underlying autoantibody associated pathomechanisms contribute to the variability of clinical presentations, is so far not well understood. In this study, we analyzed binding patterns of GlyR autoantibodies from patients grouped to their disease SPS, PERM or epilepsy to murine central nervous system (CNS) tissues. Serum samples were first evaluated for autoantibody binding on transfected HEK-293 cells, murine spinal cord, and brain tissue samples. Autoantibody binding was further verified by co-localization with commercial antibodies against different GlyR subunits and the synaptic marker synaptophysin. We found several patterns distinguishing the different syndromes: 1. Specific GlyR binding in the grey matter of the spinal cord was only present in patients with SPS and PERM. 2. Only for PERM patients, all sera bound to all three layers of the hippocampus, 3. Only serum from the epilepsy patients distinctly bound to the subiculum. Most CNS regions of enhanced autoantibody accumulation however varied among the groups of SPS, PERM and epilepsy patients and also within groups. Overall, GlyR directed autoantibodies bound GlyR-rich areas of the CNS. Even if variations between labeled areas and cell layers were rather patient-specific than group-specific, functionality of the labeled areas aligned with the patients' individual symptoms. Labeled areas and cell layers differing between patients could thereby explain the variability of symptoms between and within the diseases. The observed diversity suggests a necessity for a personalized

approach correlating patient-specific autoantibody binding patterns, functional properties, phenotype, and treatment approaches.

Disclosures: A. Wiessler: None. **I. Piro:** None. **E. Tuzun:** None. **C.L. Sommer:** None. **C. Villmann:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.19/B58

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

Support: NIH Grant

Title: Modeling Neurodevelopmental Deficits Caused by GABAA Receptor Mutations in DEEs Using Human iPSCs

Authors: *B. DE FREITAS BRENHA[1], M. WILLIAMS[2], T. BENSKE[3], T. MU[4];
[1]Case Western Univ., Cleveland, OH; [2]CASE WESTERN RESERVE UNIVERSITY, Cleveland, FL; [3]Case Western Reserve Univ., CLEVELAND, OH; [4]Case Western Reserve Univ., Cleveland, OH

Abstract: Developmental and epileptic encephalopathies (DEEs), the most severe epilepsy syndromes, are characterized by early-onset seizures, intellectual disability, and psychiatric comorbidities. Seizures in DEEs are typically drug-resistant, highlighting the need to uncover cellular mechanisms underlying these disorders and identify new therapeutic strategies. Genes encoding ion channels, including $GABA_A$ receptor ($GABA_AR$) subunits, are most frequently implicated in DEEs. Traditionally, loss of function (LOF) variants in $GABA_AR$ subunits, lead to decreased neuronal inhibition resulting in many DEE phenotypes. However, $GABA_AR$ mediate depolarizing membrane responses in the immature brain playing an essential role for cortical development, which are critical for neuronal differentiation, migration, neurite outgrowth, and synapse formation. We hypothesize that the LOF $GABA_AR$ subunits mutations lead to weakened GABA-induced depolarization during early development. Using human iPSC-derived cortical neurons, we find that mutant neurons exhibit reduced NKCC1 expression, altered chloride homeostasis and increased immature neuronal maker (Ngn1 and DCX) along with downregulated mature neuronal markers (MAP2 and Tuj1), consistent with delayed neuronal differentiation and cytoskeletal instability. Functional assays using multielectrode arrays (MEAs) reveal that control neurons exhibit more robust spontaneous firing activity compared to mutant lines, supporting the role of early GABAergic signaling in shaping network maturation. Additionally, we are investigating these findings in brain organoids, providing a more complex model to explore neuronal migration and network formation. This is the first study to model $GABA_AR$ mutations in a human neuronal environment, providing a novel platform to explore

unidentified molecular mechanisms in $GABA_AR$-related disorders opening new avenues for therapeutic intervention.

Disclosures: B. de Freitas Brenha: None. **M. Williams:** None. **T. Benske:** None. **T. Mu:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.20/B59

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

Support: NIH RO1 NS112534

Title: Membrane mediated activation of GABAAR by general anesthetics

Authors: *S. B. HANSEN[1], Z. YUAN[2];
[1]Inst. for Med. Physiol., Chinese Inst. for Med. Res., Beijing, China; [2]molecular medicine, The Scripps Res. Inst., Jupiter, FL

Abstract: Gamma-aminobutyric acid type A receptors ($GABA_ARs$) are key targets of general anesthetics. While anesthetics binding sites on $GABA_ARs$ are well-studied, the role of the surrounding lipid environment remains poorly understood. Recently, we showed that $GABA_ARs$ activation requires translocation from GM1 rafts to PIP_2 domains, prompting the involvement of anesthetics in this process. Using super-resolution microscopy in primary cortical neurons, we demonstrate that propofol, an intravenous anesthetic, reduces cellular cholesterol levels and induces $GABA_AR$ translocation to PIP_2 domains. Volatile anesthetics, isoflurane and chloroform, also facilitate $GABA_AR$ translocation but increase cholesterol levels and enlarge GM1 rafts. Anti-malarial drugs, including hydroxychloroquine and artemisinin, similarly, alter $GABA_AR$ localization. Experiments with bicuculline, a GABAARs antagonist, revealed that receptor conformational changes, rather than mere raft disruption, are necessary for translocation. The specificity of propofol's action was confirmed using its non-anesthetic analog, 2,6-di-tert-butylphenol, which neither disrupted lipid rafts nor altered $GABA_AR$ localization. These findings highlight the involvement of lipids in anesthetic action and bridge the gap between the protein and membrane mechanisms in understanding of $GABA_ARs$ regulation.

Disclosures: S.B. Hansen: None. **Z. Yuan:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.21/B60

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

Support: NIH RO1 NS112534

Title: F6 is an atypical anesthetic in flies

Authors: *J. ZHANG[1], S. B. HANSEN[2];
[1]Chinese Institues For Med. Research, Beijing, Beijing, China; [2]Chinese Inst. for Med. Res., Beijing, China

Abstract: Volatile anesthetics (VA) are a collection of diverse hydrophobic molecules that reverse loss of consciousness in animals. For almost all VA, their potency is dictated by hydrophobicity not their chemical shape, an observation known as the Meyer-Overton correlation. One exception, 1,2-dichlorohexafluorocyclobutane (F6, 2N), known as a non-immobilizer, causes some anesthetic like behaviors in rodents, including amnesia and analgesia, but no immobilization and not at expected concentration. Previously we showed F6 did not disrupt lipid raft function at concentrations predicted by Meyer-Overton, thus helping to establish a membrane mediated mechanism of anesthesia. Here we use show F6 causes anesthesia in flies at concentration 6x times that expected of Meyer-Overton, suggesting F6 is an atypical anesthetic. Using cultured cells, we show the F6 concentrations that cause anesthesia also competes with palmitate, causing gamma amino butyric acid type A receptor (GABAAR) and phospholipase D2 enzyme (PLD2) to leave ordered GM1 lipid domains and bind to signaling lipid phosphatidylinositol 4,5 bisphosphate (PIP2) 200 nm away. We conclude F6 anesthesia utilizes a membrane mediated mechanism.

Disclosures: J. Zhang: None. **S.B. Hansen:** None.

Poster

PSTR373: Ionotropic Glutamate, GABA, and Glycice Receptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.22/C1

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

Title: Use of neuronal mitochondrial isolation to assess NDUFS4 levels in GABAergic neurons

Authors: *L. LOPEZ[1], B. KUUSIK[3], C. VOISINE[2], D. S. MITHAL[3];
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Abstract: GABAergic neurons play a crucial role in the functioning of the central nervous system. GABAergic neurons balance excitation and inhibition in the brain and require proper mitochondrial function to sustain neural circuitry. The NDUFS4 protein is one of the 45 subunits in mitochondrial Complex I of the electron transport chain (ETC). The ETC is responsible for

energy production, yet in mice deletion of the NDUFS4 subunit in the brain is sufficient to impair neural control and disrupt cognitive and motor function. More specifically, studies have shown that selective deletion of NDUFS4 restricted to GABAergic neurons causes impaired inhibitory control and widespread neuronal dysfunction resulting in seizures in mice. However, the mechanisms that allow NDUFS4 deletion to alter mitochondrial function in GABAergic neurons are unclear. One possibility is that the absence of NDUFS4 causes compensatory changes in the protein structure of mitochondrial Complex I or in other mitochondrial compartments. By using novel mitochondrial isolation techniques from the brain, the project aims to compare mitochondrial protein composition from GABAergic neurons in the presence and absence of NDUFS4. The study will advance our understanding of mitochondria in GABAergic neurons and potentially lead to the development of biomarkers, targeted therapies, and personalized treatments for mitochondrial diseases.

Disclosures: L. Lopez: None. **B. Kuusik:** None. **C. Voisine:** None. **D.S. Mithal:** None.

Poster

PSTR374: Voltage-Gated Calcium Channels

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR374.01/C2

Topic: B.03. Ion Channels

Title: Intracellular transport of PSD-95 is impaired by the $Ca_v3.1$ mutation causing SCA42.

Authors: *Y. TAGANE[1], M. NAKAMORI[2], H. MORINO[3], H. MARUYAMA[2];
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Abstract: Mutations in *CACNA1G*, encoding the T-type calcium channel $Ca_v3.1$, have been identified as the genetic cause of spinocerebellar ataxia type 42 (SCA42). Previously, we demonstrated that the voltage-dependence of $Ca_v3.1$ activation shifted toward depolarizing in HEK293T cells expressing the mutant $Ca_v3.1$, and that this shift was reversed by zonisamide. Downregulation of $Ca_v3.1$ has also been reported in other SCA types, including SCA1, SCA2, and SCA7, suggesting that $Ca_v3.1$ regulation is associated with a broader range of pathogenesis of spinocerebellar degeneration. T-type calcium channels are involved in long-term potentiation and synaptic plasticity. Therefore, we investigated whether $Ca_v3.1$ dysfunction could affect synaptogenesis. We focused on PSD-95, a postsynaptic scaffolding protein essential for synapse formation. In a preliminary study using Neuro-2a cells, transient expression of mutant $Ca_v3.1$ led to aberrant localization of PSD-95 in the neuronal soma, accompanied by the formation of large puncta. In the current study, we established stable SH-SY5Y cell lines expressing either wild-type or mutant $Ca_v3.1$ using the piggyBac transposon system. These cells were transfected with PSD-95.FingR-GFP, a GFP-tagged intrabody that specifically binds endogenous PSD-95, allowing live imaging of intracellular transport dynamics. In the cells expressing wild-type

$Ca_v3.1$, PSD-95 was efficiently transported from the soma to dendrites. Conversely, in the cells expressing mutant $Ca_v3.1$, PSD-95 moved back and forth between the soma and dentrites. However, the velocity of PSD-95 movement within dendrites did not differ significantly between wild-type and mutant expressing cells. When a T-type calcium channel inhibitor zonisamide was added to the culture medium, the dendritic localization of PSD-95 was improved in mutant-expressing cells. These findings indicate that $Ca_v3.1$ regulates intracellular transport of a synaptic protein through local calcium signaling, and that the mutation in $Ca_v3.1$ disrupts this process. The improvement in PSD-95 intracellular transport dysfunction by zonisamide may be attributed to the shift in the voltage-dependent properties of mutated calcium channels induced by zonisamide, which improves local calcium currents in neurons. The observation that pharmacological intervention ameliorates transport dynamics suggests that our experimental system is useful platform for evaluating candidate therapeutics targeting intracellular transport mechanisms in spinocerebellar degeneration.

Disclosures: Y. Tagane: None. **M. Nakamori:** None. **H. Morino:** None. **H. Maruyama:** None.

Poster

PSTR374: Voltage-Gated Calcium Channels

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR374.02/C3

Topic: B.03. Ion Channels

Support: MH115045
Center for Psychiatric Research

Title: Functional Consequences of Disease-Associated CACNA1G Missense Variants in Schizophrenia, Epilepsy, and Neurodevelopmental Delay

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Abstract: CACNA1G encodes the low-voltage-activated CaV3.1 T-type calcium channel, which is abundantly expressed in Purkinje neurons of the cerebellum and widely distributed throughout the thalamus and cortex. CaV3.1 channels play essential roles in sleep stabilization, cognitive function, and motor memory. While exome sequencing studies have implicated CACNA1G in schizophrenia, epilepsy, and neurodevelopmental delay, the specific molecular phenotypes that contribute to these disorders remain unclear. In this study, we analyzed coding variants of CACNA1G located within three hotspot regions of the CaV3.1 channel, encompassing variants significantly enriched in disease populations as well as those found in population controls from gnomAD. Using established automated patch-clamp protocols (SyncroPatch384), we functionally evaluated a series 84 CACNA1G variants, comparing the biophysical properties of

variant-encoded channels across the schizophrenia (SCHEMA), epilepsy (Epi25), and population control cohorts. Biophysical parameters assessed included whole-cell current density, voltage-dependent activation and inactivation, and inactivation/deactivation kinetics. Our comparative analyses revealed that cells expressing schizophrenia-associated variants exhibited significantly reduced peak current densities, whereas epilepsy-associated variants demonstrated a significant leftward shift in voltage-dependent activation. Notably, variants from patients with neurodevelopmental delays showed pronounced slowing of inactivation kinetics. Clinical data from a subset of patients further supported the observation that seizure-associated variants frequently cause a leftward shift in voltage-dependent activation, suggesting that such shifts in CaV3.1 gating may underlie epilepsy. Additionally, similar shifts were also observed in patients with neurodevelopmental delays. Overall, these functional characterizations of an allelic series of CACNA1G variants across disease and control cohorts highlight distinct channel phenotypes that may contribute to specific disease pathophysiology in humans.

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Poster

PSTR374: Voltage-Gated Calcium Channels

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Topic: B.03. Ion Channels

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Title: Targeting N-Glycosylation to Reverse Pathogenic Gain-of-Function in CACNA1A Channelopathies: Therapeutic Potential of MPI Inhibition

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Abstract: Mutations in the *CACNA1A* gene, encoding the α_{1A} subunit of the $Ca_V2.1$ (P/Q-type) voltage-gated calcium channel, are responsible for a range of neurological disorders, including Familial Hemiplegic Migraine type 1 (FHM1) and Congenital Ataxia (CA). These mutations mainly induce gain-of-function effects that increase calcium influx and neuronal excitability, facilitating the initiation and propagation of cortical spreading depression (a key process in migraine pathophysiology) and triggering cerebellar Purkinje cell hyperexcitability and irregular firing patterns (leading to ataxia). Hypoglycosylation of $Ca_V2.1$ subunits, as observed in Phosphomannomutase 2 deficiency (PMM2-CDG), can mimic these pathogenic gain-of-function effects and contribute to overlapping clinical phenotypes. Given that Phosphomannose Isomerase (MPI) competes with PMM2 for mannose-6-phosphate, we hypothesized that pharmacological

inhibition of MPI, combined with mannose supplementation, could enhance N-glycosylation of $Ca_V2.1$ channels and mitigate the gain-of-function associated with specific *CACNA1A* mutations. We investigated this strategy using HEK293 cells expressing wild-type or mutant (Y1245C, ΔF1502) $Ca_V2.1$ channels. Cells were treated with the MPI inhibitor MLS0315771 and mannose, followed by assessment of N-glycosylation (western blot) and channel function (whole-cell patch-clamp electrophysiology). MLS0315771 with mannose increased N-glycosylation of the $\alpha_2\delta$ subunit. The ΔF1502 mutation (linked to FHM1 and CA) induced a pronounced leftward shift in activation voltage (by ~26 mV, $p<0.01$, n=3-13, Mann-Whitney Test) and slowed inactivation kinetics ($p<0.05$, n=5-11, Mann-Whitney Test), both hallmarks of pathological gain-of-function. Remarkably, MPI inhibition with mannose partially reversed these effects, normalizing activation threshold (by ~6.5 mV, $p<0.05$, n=4, Mann-Whitney Test) and inactivation kinetics ($p<0.05$, n=4-5, Mann-Whitney Test). The Y1245C mutation (linked to FHM1) only reduced voltage activation threshold (by ~6 mV, $p<0.05$, n=7-13, t test), but the treatment with MLS0315771 and mannose did not modify such gain-of-function.
Our results demonstrate that pharmacological enhancement of N-glycosylation via MPI inhibition selectively counteracts the pathogenic gain-of-function of certain *CACNA1A* mutations, particularly ΔF1502. This approach represents a promising targeted therapy for $Ca_V2.1$-related channelopathies, including FHM1 and CA, and highlights the therapeutic potential of modulating post-translational channel modifications in genetic neurological disorders.

Disclosures: G. Rodriguez Urquirizar: None. **A. Edo-Pérez:** None. **J. Fernandez-Fernandez:** None.

Poster

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Title: Altered gating of two $Ca_V2.1$ variants linked to neurodevelopmental disorders with epilepsy and migraine

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Abstract: P/Q-type ($Ca_V2.1$) Ca^{2+} channels regulate the release of neurotransmitter at central synapses. Missense and nonsense mutations in *CACNA1A*, the gene which encodes the principal α_{1A} subunit of the $Ca_V2.1$ channel complex, are well-known to cause Episodic Ataxia type 2 (EA2) and Familial Hemiplegic Migraine type 1 (FHM1). These $Ca_V2.1$ channelopathies are typically caused by either channel loss/reduction-of-function (LOF) or gain-of-function (GOF), respectively. However, recent genome-wide sequencing has revealed that point mutations in $Ca_V2.1$, in fact, underlie a spectrum of neurological disorders which feature epilepsy, tremor, nystagmus, hypotonia, cerebellar atrophy, cognition deficits and global developmental delay. Given the multiple manifestations of the mutations and the broad range of severity amongst these disorders, the assessment of the impact of an individual pathological mutation on channel function is essential for understanding the aetiology of a given case. To this end, we expressed one newly-identified and a previously-reported, but yet-to-be characterized, $Ca_V2.1$ variant (V176M and R1673C, respectively) in HEK 293 cells and investigated their biophysical properties using patch-clamp electrophysiology. Both mutations had multiple effects on channel function, though each mutation affected channel gating differently. V176M displayed a ~10 mV hyperpolarizing shift in activation and slowed deactivation. By contrast, the R1673C variant slowed channel activation kinetics, significantly delayed closure and accelerated recovery from inactivation. Molecular modelling revealed structural alterations in the two variants which may account for the observed changes in channel gating. Taken together, our results indicate that the V176M and R1673C variants likely cause severe $Ca_V2.1$ channelopathies through multiple, distinct mechanisms.

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Poster

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Topic: B.03. Ion Channels

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Title: Lobe-specific cerebellar histopathology in α2δ2 knockout mice

Authors: *E. C. JEROME[1], A. A. GALLAGHER[1], A. J. ELLINGSON[2], E. SCHNELL[1]; [1]Anesthesiol. and Perioperative Med., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Neurosci. Grad. Program, OHSU, Portland, OR

Abstract: Voltage gated calcium channels (VGCCs) allow for depolarization-induced calcium entry into neurons and are crucial regulators of neuronal signal transduction. The CACNA2D family of channel subunit proteins contribute to VGCC trafficking and coupling to downstream effector mechanisms. Mutation of one of the CACNA2D isoforms, α2δ-2, causes a range of neurological outcomes including epilepsy and ataxia in both humans and mice. α2δ-2 is highly expressed in cerebellar Purkinje cells, although the specific contribution of cerebellar dysfunction to neurological phenotypes has yet to be elucidated. Using immunohistochemical staining and confocal microscopy, we are examining morphological and molecular markers in cerebellar Purkinje cells from wildtype and α2δ-2 knockout (KO) mice to illuminate possible cerebellar circuit abnormalities. We observe a dramatic lobe-specific increase in Purkinje cell tyrosine hydroxylase expression in α2δ-2 KO mice when compared to their wildtype counterparts. Although this hints at a potential disturbance in cerebellar dopamine signaling, other proteins typically required for dopaminergic signaling do not appear to be present in Purkinje cells based on histochemical staining. Ongoing work aims to determine whether other known histopathological changes in Purkinje cells, such as altered climbing fiber terminal localization or Purkinje cell degeneration also vary in a lobe-specific manner. Together, we hope to determine the etiology of lobe-specific changes in cerebellar function with the intent of better understanding the roles of α2δ-2 in Purkinje cells as well other α2δ isoforms throughout the brain.

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Poster

PSTR374: Voltage-Gated Calcium Channels

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Program #/Poster #: PSTR374.06/C6

Topic: B.03. Ion Channels

Support: NIH Grant NS055251

Title: Developmentally-regulated splicing of cacna1a in cerebellum

Authors: *A. SALAZAR[1], M. S. SISTI[2], D. LIPSCOMBE[3];
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Abstract: Cell-specific alternative splicing is critical for normal cell function. The *Cacna1* gene encodes a family of $Ca_V2.1$ proteins which are upregulated during development including in cerebellum where it controls short term synaptic plasticity, synaptic transmission and synapse refinement. Human disease-causing mutations in *Cacna1a* are linked to ataxia, cerebellar atrophy, familial hemiplegic migraine, cognitive impairment, and developmental disabilities. Purkinje cells of the cerebellum are enriched in $Ca_V2.1$ channels. *Cacna1a* splice isoforms of $Ca_V2.1$ have different proximal C-termini which alter their sensitivity to Ca-Calmodulin

(CaCaM) -dependent channel inactivation. Building from published studies, we find a major switch in these *Cacna1a* mRNAs, from e37b-containing to e37a-containing, in mouse cerebellum during the first 4 weeks of life (eg. Chaudhuri et al., *JNeuroSci*, 2005). There are no pharmacological tools available that can discriminate e37a-$Ca_V2.1$ and e37b-$Ca_V2.1$ protein function so we generated two mouse models with restricted *Cacna1a* splice options: e37a-only ($e37^{aa*/aa*}$) and e37b-only ($e37^{b*b/b*b}$) mice to study the functional importance of this splice site in *Cacna1a*. $Ca_V2.1$ channel protein was reduced in brains of e37a-only and e37b-only mice, complicating our ability to attribute in vivo phenotypes to changes in splices from reduced protein expression. However, our behavioral analyses show that e37b-only mice exhibit severe progressive ataxia, resulting in premature death at weaning (by ~P21). By contrast, e37a-only mice exhibit only mild ataxia and survive into adulthood. Our preliminary studies suggest that the $Ca_V2.1$-e37a splice isoform is essential for normal cerebellum development and function and for viability. Anatomically, the overall structure of the cerebellum, including the presence and density of Purkinje cells appears to be normal in preliminary analysis of e37a-only and e37b-only mice at P21, suggesting that the $Ca_V2.1$-e37a splice isoform may be playing a role in synaptic refinement and function. We are currently exploring this, and other hypotheses to establish which features of $Ca_V2.1$-e37a are essential for its function in cerebellum including Ca-Calmodulin (CaCaM) -dependent channel inactivation, and if other regions of the nervous system are dependent on developmentally regulated splicing of *Cacna1a*.

Disclosures: A. Salazar: None. **M.S. Sisti:** None. **D. Lipscombe:** None.

Poster

PSTR374: Voltage-Gated Calcium Channels

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Topic: B.03. Ion Channels

Support: SECIHTY PhD Scholarship No. 29713

Title: In Silico and Electrophysiological Characterization of a Novel O1-Conotoxin s-xm6a from Conasprella ximenes with Antinociceptive Effect

Authors: *J. LÓPEZ-CARRILLO[1], F. MERCADO[3], A. ALMANZA[3], J. GASPERIN-BULBARELA[1], N. CARAM-SALAS[1], A. LICEA[2];
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Abstract: Chronic pain affects 35-55% of the global population, posing a significant clinical and therapeutic challenge. Conotoxins, bioactive peptides derived from cone snail venom, modulate nociceptive signaling through their high selectivity for voltage-gated ion channels (VGICs), offering promising alternatives to opioid-based therapies. We recently identified s-xm6a, a novel

O1-superfamily conotoxin from *Conasprella ximenes*, which demonstrated potent antinociceptive effects *in vivo*. To elucidate its mechanism of action, we predicted its three-dimensional structure and assessed its impact on voltage-activated calcium channels using electrophysiological and molecular docking approaches. Utilizing ColabFold v1.5.5, we modeled s-xm6a, refined it with 500 ns molecular dynamics (MD) simulations in NAMD2 with the CHARMM36 force field, and validated its stability through RMSD and clustering analysis. The peptide exhibited an inhibitory cystine knot (ICK) fold stabilized by three disulfide bridges, maintaining a compact structure throughout the simulation (mean RMSD: 0.90 ± 0.22 Å). Electrophysiological recordings in dorsal root ganglion (DRG) neurons revealed that s-xm6a, tested at seven concentrations (0.0001-10 μM), reduced total voltage-activated calcium current (ICa) in a concentration-dependent manner (one-way ANOVA, $F_{(7, 40)} = 17.87$, $p<0.05$; n = 6 cells per concentration). Maximal reduction of ICa occurred at 3-10 μM to 64.49 ± 4.36% and 62.27 ± 3.39% of control levels, respectively, and the dose-response curve revealed a biphasic IC_{50} profile (20 nM and 237 nM), suggesting an interaction with multiple channel subtypes. Ongoing molecular docking studies with N-type calcium channels aim to define key binding interfaces. Collectively, our findings provide structural and functional insights into s-xm6a and support its potential as a novel analgesic candidate targeting the calcium channels involved in nociceptive processing.

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Poster

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Title: Cre-dependent Cacna1b inactivation reveals cell-specific functions of $Ca_V2.2$ channels in Trpv1-lineage neurons in vivo

Authors: *M. S. SISTI[1], R. Y. MEIR[2], D. LIPSCOMBE[3];
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Abstract: Voltage-gated $Ca_V2.2$ channels have a dual role in DRG neurons; they support presynaptic transmitter release in the dorsal horn spinal cord, and control neuroimmune signalling in skin. Using pharmacological inhibition and global *Cacna1b* knockout mice (*Cacna1b*$^{-/-}$, gKO) our lab has shown that $Ca_V2.2$ channels in skin mediate capsaicin and CFA-induced heat hypersensitivity associated with neuroinflammation. To establish definitively if *Trpv1*-lineage neurons are the source of $Ca_V2.2$ channels in these models of inflammation, we inactivated *Cacna1b* in *Trpv1*-lineage neurons using a cre-dependent strategy,

Cacna1b$^{fl/fl}$/*Trpv1*$^{Cre/-}$ (cKO). We validated the cKO mouse model by showing: reduced $Ca_V2.2$ protein levels in DRG, but not in brain; loss of $Ca_V2.2$ currents in *Trpv1*-expressing DRG neurons of cKO mice as compared to WT controls; and the absence of a hyperactivity phenotype which is characteristic of gKO mice. *Trpv1* is expressed in both heat and cold sensing neurons but not in mechanoreceptors. The latency to paw withdrawal to heat and cold stimuli were longer in cKO mice, whereas responses in cKO mice to mechanical stimuli were indistinguishable from WT. In addition to validating *Trpv1*-dependent inactivation of *Cacna1b*, our findings show that $Ca_V2.2$ channels regulate signaling in cold sensing neurons. After validation, we used cKO mice to show that *Trpv1*-lineage neurons are the sole source of $Ca_V2.2$ supporting heat hypersensitivity in capsaicin-induced inflammation, but that $Ca_V2.2$ channels expressed in both *Trpv1*-lineage and non *Trpv1*-lineage neurons mediate heat hypersensitivity that develops during CFA-induced prolonged inflammation. Finally, we assessed features of *Trpv1*-lineage neuron excitability in cKO mice compared to WT controls including: membrane potential, rheobase, action potential (AP) trajectory, and AP firing frequency. To our surprise, *Trpv1*-lineage neurons from cKO mice were hyperexcitable compared to WT; APs were elicited by smaller currents, the peaks were higher, and they fired at higher frequencies compared to WT, without differences in membrane potentials or cell input resistance. Our data point to a compensatory increase in the density of Na_V ion channels in *Trpv1*-lineage neurons of cKO mice, perhaps in response to reduced synaptic efficacy. Supporting this hypothesis, we show that acute inhibition of $Ca_V2.2$, by direct application of w-conotoxin GVIA, has no effect on excitability of *Trpv1*-lineage neurons of WT mice. Our studies suggest that significant compensatory changes in the expression of ion channels and the excitability of neurons KO mouse models without any overt changes in behavior.

Disclosures: M.S. Sisti: None. **R.Y. Meir:** None. **D. Lipscombe:** None.

Poster

PSTR374: Voltage-Gated Calcium Channels

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Topic: B.03. Ion Channels

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5T32NS007466-25

Title: α2δ-2 functionally couples calcium entry to synaptic gaba release from pv+ interneurons onto hippocampal granule cells

Authors: *A. J. ELLINGSON[1], A. A. GALLAGHER[1], E. C. JEROME[1], E. SCHNELL[1,2]; [1]OHSU, Portland, OR; [2]Portland VA Healthcare, Portland, OR

Abstract: Voltage gated calcium channels (VGCCs) are required for many aspects of neuronal signal transduction, including action potential-dependent synaptic transmission. VGCCs consist of several different subunits, including both pore-forming α1 channels and auxiliary modulatory subunits such as β, γ, and α2δ proteins. α2δ subunits contribute to surface and subcellular trafficking of VGCCs, and mutations in genes that encode for any of the three brain α2δ isoforms cause neuronal dysfunction. Previous studies of α2δ isoforms in genetic mouse models have been complicated by isoform compensation and the mortality of double and triple knockout mutants. However, the α2δ-2 isoform is selectively expressed in parvalbumin (PV) positive neurons throughout the hippocampus, and loss of this isoform causes epilepsy in both mice and humans. To determine how α2δ-2 loss affects PV+ interneuron function, we studied synaptic function in α2δ-2 WT and KO mice selectively expressing Channelrhodpsin2 in PV+ neurons. We have found that loss of α2δ-2 in PV+ interneurons significantly decreases synaptic transmission from PV+ interneurons, primarily through a reduction in the probability of release of GABA-containing vesicles. Ongoing studies are examining other aspects of synaptic transmission between PV+ interneurons and hippocampal dentate granule cells, as well as potential changes in the intrinsic properties of PV+ neurons. Together, our preliminary data suggest that α2δ-2 is important for functionally coupling VGCCs to the presynaptic vesicular release machinery in PV+ cells, which might have important implications for our understanding of the epileptic phenotype in these mice.

Disclosures: A.J. Ellingson: None. **A.A. Gallagher:** None. **E.C. Jerome:** None. **E. Schnell:** None.

Poster

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Topic: B.03. Ion Channels

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DFG Grant RY117/3-3

Title: Alternative Cav2 splice isoforms mediate fast versus graded synaptic transmission in Drosophila

Authors: *T. RINAS[1], V. PAMPANIN[1], L. KILO[2], C. DUCH[1], S. RYGLEWSKI[1];
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Abstract: In vertebrate central synapses, fast action potential (AP) triggered transmitter release is predominantly mediated by Cav2.1 and Cav2.2 channels, while graded synaptic transmission is guaranteed by specialized active zones and L-type Cav1 channels. To accommodate these different modes of neurotransmission, these calcium channels exhibit different biophysical

properties: Cav2 channels open and close relatively fast, while Cav1 channels open fast and then remain open for a longer time. By contrast, in Drosophila both, fast as well as graded synaptic transmission are mediated by the only Cav2 homolog, Cacophony (Cac). Therefore, in Drosophia, Cav2 channels have to fulfil the physiological requirements to induce transmitter release as triggered by APs but also for graded transmitter release where the number of released vesicles is linearly correlated with the grade of depolarization. The *cac* gene is subject to heavy alternative splicing. Alternative splicing of mutually exclusive exon pairs of Cac affects channel properties such as activation voltage and channel localization at the active zone (AZ) (Bell et al., eLife, 2025). By using CRISPR/Cas9, we genomically excised one of two mutually exclusive exons of the exon pair *IS4A* or *IS4B*, that encodes the fourth transmembrane segment (S4) of the first homologous domain (I), that is part of the voltage sensor. Using immunohistochemistry, electrophysiology, and behavioral experiments, we find that Cac isoforms with exon IS4B mediate transmission at the NMJ but do not trigger release at the photoreceptor to lamina graded synapse. *Vice versa*, Cac isoforms containing alternative exon IS4A fail to induce vesicle release at the NMJ but are necessary for transmission at the graded photoreceptor to lamina synapse. We propose that differential expression of Cac isoforms with either one or the other mutually exclusive exon IS4A or IS4B provides functional diversity of Cacophony suited for fast versus graded synapses in Drosophila.

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Poster

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Topic: B.03. Ion Channels

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DFG RY117/4-1

Title: Calcium channel gating property variance safeguards neuronal excitability

Authors: S. HILGERT[1], S. C. RYGLEWSKI[2], ***C. DUCH**[3];
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Abstract: Neural circuits are composed of different types of neurons, each of which exhibits distinctly different computational properties that result from the sets of ion channels a neuron expresses. Profound insight exists into how neural computations arise from the precise regulation of ion channels, how different combinations of channels can support similar computations, and how channel properties are modulated. However, most neurons express a much larger number of

ion channel types than required in models to adequately tune membrane excitability. The reason for the large numbers of different ion channels and their isoforms that are expressed in neurons remains incompletely understood. Here we employ an experiment-theory approach pairing electrophysiology with Drosophila genetics and mathematical modelling to show that the expression of large numbers of ion channels promotes the robustness of neuron type-specific computations. Our data show experimentally and in models that the larger the diversity of voltage gated calcium channel splice isoforms that are expressed, the larger is the resilience of neuronal excitability to perturbations of outward currents. Specifically, reducing the number splice variants of the Drosophila Cav2 homolog cacophony from 18 to 4 by on-locus exon excision significantly increases the robustness of flight motoneuron type- I membrane excitability to acute pharmacological and genetic knock-down of Shab Kv2 delayed rectifier potassium current. Experimental and mathematical evidence support the view that increased excitability robustness does not require adjustments in cacophony calcium current mean properties. Instead, increases of the variance of calcium channel gating properties that result from channel isoform diversity broaden the dynamic input range the neuron can compute without reaching depolarization block. This provides conceptually novel insight into the functional consequences of the tremendous variety and diversity of ion channels.

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Poster

PSTR374: Voltage-Gated Calcium Channels

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Topic: B.03. Ion Channels

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JSPS KAKENHI JP23K18485
JSPS KAKENHI JP23K28178

Title: Quantitative Description of Voltage-Activated Ca^{2+} Current Responsible for Graded Membrane Response in Paramecium multimicronucleatum. II. Kinetic Analysis of Transient Ca^{2+} Current

Authors: *Y. TOMINAGA, T. TOMINAGA;
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Abstract: *Paramecium multimicronucleatum* is a fundamental model organism for studying membrane potential control of cellular behavior. Voltage-activated membrane currents govern its

behavioral responses. Following our kinetic analysis of K^+ currents presented at SFN 2024, this abstract details the quantitative analysis of the transient Ca^{2+} inward current responsible for generating graded action potentials. In *Paramecium*, it was pioneeringly shown in 1978 that Ca^{2+} channels undergo inactivation in a Ca^{2+}-dependent manner, a property also found in nerve cells but mediated by pharmacologically distinct channels in *Paramecium*. Despite acknowledging this unique, Ca^{2+}-dependent inactivation, a detailed quantitative kinetic description has been previously lacking. To perform this analysis, we first isolated the Ca^{2+} current by accounting for the unique resting membrane conductance properties of *Paramecium*, including its voltage-dependent resting conductance. Using a two-step voltage clamp protocol, we found that the Ca^{2+} current undergoes complete inactivation at all potentials. This phenomenon and its time course could not be described by the traditional Hodgkin-Huxley (HH) model of channel inactivation. We successfully described the kinetic properties of this complete, Ca^{2+}-dependent inactivation by adopting the concept of open channel block, a model consistent with an open channel becoming non-conducting without reactivation. We also quantitatively analyzed the activation kinetics of the Ca^{2+} current, finding it could be characterized using a Hodgkin-Huxley type formalism. Analysis of steady-state activation and inactivation curves, as well as the time course of de-inactivation, further highlighted properties that distinguish this channel from standard HH models, such as significantly slower de-inactivation kinetics compared to inactivation. Our quantitative kinetic analysis provides a comprehensive description of the transient Ca^{2+} current in *Paramecium*, demonstrating its complete inactivation via a mechanism consistent with open channel block. This work advances our understanding of the voltage-activated currents underlying graded membrane responses and behavioral control in this established model organism.

Disclosures: Y. Tominaga: None. **T. Tominaga:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

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Program #/Poster #: PSTR375.01/C13

Topic: B.04. Synaptic Transmission

Support: Karen Toffler Charitable Trust
MBI Gator NeuroScholars
R01DA058143
R01NS071122-07A1
R21NS133384

Title: Midbrain dopamine drives splenic immunity through a brain to body circuit

Authors: *A. GOPINATH[1], A. J. APICELLA[8], A. SMITH[1], E. MILLER[1], L. PHAN[2], J. SEIBOLD[1], A. BERTERO[9], L. HORNSTEIN[1], H. CIUPE[3], D. BOOTH[1], L. WOOD[3], N. SOUNDARA[1], S. CAMPOS[1], P. TREVIZAN-BAÚ[4], L. R. REZNIKOV[5], N. URS[6], O.

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Abstract: Midbrain dopamine neurons are well-known to shape central nervous system function, yet there is growing evidence for their influence on the peripheral immune system. Here we demonstrate that midbrain dopamine neurons form a circuit to the spleen via a multi-synaptic pathway from the dorsal vagal complex (DVC) through the celiac ganglion. Midbrain dopamine neurons modulate the activity of D1-like and D2-like dopamine receptor-expressing DVC neurons. *In vivo* activation of midbrain dopamine neurons induced dopamine release in the DVC and increased immediate early gene expression in both the DVC and celiac ganglion, indicating enhanced neuronal activity. Activation of this midbrain-to-spleen circuit reduced spleen weight and decreased naive $CD4^+$ T-cell populations without affecting total T-cell numbers. These findings unveil a functional midbrain-DVC-celiac ganglion-spleen pathway, through which midbrain dopamine neurons modulate splenic immunity. These novel insights into the neural regulation of the immune system have important implications for diseases involving altered dopamine neurotransmission and highlight potential targets for immunotherapeutic interventions.

Disclosures: A. Gopinath: None. **A.J. Apicella:** None. **A. Smith:** None. **E. Miller:** None. **L. Phan:** None. **J. Seibold:** None. **A. Bertero:** None. **L. Hornstein:** None. **H. Ciupe:** None. **D. Booth:** None. **L. Wood:** None. **N. Soundara:** None. **S. Campos:** None. **P. Trevizan-Baú:** None. **L.R. Reznikov:** None. **N. Urs:** None. **O. Guryanova:** None. **D.W. Wesson:** None. **H. Khoshbouei:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.02/C14

Topic: B.04. Synaptic Transmission

Support: NIH grant GR1069105

Title: Atlas: a rationally designed protein that mediates anterograde transsynaptic tracing

Authors: *H. HUANG, J. RIVERA, H. SOHN, D. B. ARNOLD;
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Abstract: Recent advancements in virus-based neural tracing techniques have provided new opportunities to elucidate neuronal circuits. However, these techniques, which rely on the intrinsic properties of viruses, have multiple drawbacks, including toxicity, lack of specificity,

and difficulties in handling. In addition, the mechanisms by which these viruses mediate transneuronal labeling remain elusive, preventing further improvement. Herein, we introduce ATLAS (Anterograde Transsynaptic Label based on Antibody-like Sensors), a rationally designed protein capable of anterograde transsynaptic labeling. ATLAS encompasses an antibody-like protein that specifically recognizes the AMPA receptor subunit GluA1 (AMPA.FingR). Following expression of ATLAS in presynaptic neurons, the AMPA.FingR and its payload, which can include Cre recombinase, are released from presynaptic sites into the synaptic cleft, after which it binds to GluA1, enters postsynaptic cells through endocytosis, and subsequently carries its payload to the nucleus. Testing in dissociate cultures verifies the presynaptic release, postsynaptic binding, and endocytosis of the ATLAS tracer. Further studies in mice show that ATLAS can mediate monosynaptic tracing *in vivo* from random or genetically determined cells that transsyneuronal tracing by ATLAS is strictly anterograde, synaptic, and non-toxic. Moreover, ATLAS exhibits activity dependence and can mediate transsynaptic tracing in both mice and rats. Its versatile modular design also allows for modification to map and manipulate neural circuits mediated by many different neurotransmitters and neuromodulators, including, potentially, circuits that have previously been refractory to mapping using viral methods.

Disclosures: H. Huang: None. **J. Rivera:** None. **H. Sohn:** None. **D.B. Arnold:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.03/C15

Topic: B.04. Synaptic Transmission

Support: NIH-NINDS R01NS130759
AEI (Spain) PCI2022-135040-2

Title: Modeling using super resolution images suggest anisotropic and anomalous diffusion in the hippocampal extracellular space

Authors: R. T. SHAKYA[1], P. GIMENEZ MINGUEZ[3], E. OLIVARES[4], J. TØNNESEN[5], ***F. SANTAMARIA**[2];
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Abstract: The extracellular space (ECS) of the brain is a highly complex and dynamic environment that plays a crucial role in regulating the transport of ions, metabolites and neurotransmitters. Diffusion is the primary mechanism of molecular transport within the ECS and thus, it is important to understand how structural and functional changes in the ECS affect

diffusion at scales relevant for neuronal function. Here, we investigate nanoscale diffusion dynamics in the ECS using super resolution shadow imaging (SUSHI) of live mouse brain slices, which enables direct visualization of the ECS architecture with high spatial resolution. We perform both Monte Carlo (MC) and diffusion equation (DifFlux) simulations of neurotransmitter movement in 2D and 3D ECS images to examine the effects of ECS heterogeneity on diffusion variability and anisotropy. We show that the ECS significantly influences extracellular signal propagation and can facilitate synaptic crosstalk across dendritic trees. Additionally, we observe the emergence of anomalous diffusion at small spatial and temporal scales, highlighting the complex nature of molecular transport in the brain's ECS.

Disclosures: R.T. Shakya: None. **P. Gimenez Minguez:** None. **E. Olivares:** None. **J. Tønnesen:** None. **F. Santamaria:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.04/C16

Topic: B.04. Synaptic Transmission

Support: Max Planck Society

Title: Increased excitatory drive to somatostatin neurons in lateral amygdala of NLGN3R451C mouse model of autism spectrum disorder

Authors: *C. VON-WALTER[1], C. T. UNAL[2], B. UNAL[2], R. M. AIKEN[1], M. BOLTON[1]; [1]Max Planck Florida Inst. for Neurosci., Jupiter, FL; [2]Canakkale Onsekiz Mart Univ., Canakkale, Turkey

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social deficits, language impairment and repetitive behavioral patterns. The underlying changes in brain function resulting in ASD are not well understood. Neuroligin 3 (NLGN3) is a post synaptic adhesion molecule that binds across the synaptic cleft to its presynaptic neurexin partner to regulate synaptic function at both excitatory and inhibitory synapses. The R451C mutation in NLGN3 was identified in 2003 in 2 brothers with ASD (Jamain et al. 2003) and subsequently over 30 mutations in the NLGN3 gene have been identified in ASD patients. The NLGN3R451C mouse model displays ASD associated behaviors such as social deficits, repetitive behavior, fear generalization and increased aggression. Given that the proper functioning of the amygdala is imperative for learning contingencies among emotionally charged stimuli and generation of adaptive emotional and social responses, we investigated amygdala circuit function in the NLGN3R451C mouse model of autism. In patch-clamp recordings in brain slices, we found that the principal neurons of the lateral amygdala (LA) were hyper-connected by feedback inhibitory networks. Given that the subset of somatostatin (SST) interneurons with a low threshold spiking intrinsic phenotype participate in feedback inhibition in LA (Ünal et al. 2020), we investigated

the connections between SST interneurons and principal neurons in BLA. We used a combination of paired recordings, and optogenetic terminal stimulation to evaluate excitatory drive from principal neurons to SST neurons and the inhibitory connection from SST neurons to principal neurons in BLA. The frequency of miniature excitatory post synaptic currents onto somatostatin interneurons was increased, supporting the hypothesis of increased excitatory drive to these neurons. In addition, the probability of connection from LA principal neuron to inhibitory neurons was increased in paired recordings. Recordings from SST neurons during optogenetic terminal stimulation of BLA principal neurons revealed an increase in the amplitude of the monosynaptic input from BLA principal neurons to SST neurons in the NLGN3R451C mice. In addition, this connection had an increase in the asynchronous component of glutamate release. The increase in the strength and the extended time course of glutamate release will likely enhance the firing properties of SST neurons. Given the role of somatostatin interneurons in regulating synaptic integration and plasticity at distal dendrites, the increased power of these neurons is likely to affect amygdala dependent learning and behavior.

Disclosures: C. Von-Walter: None. **C.T. Unal:** None. **B. Unal:** None. **R.M. Aiken:** None. **M. Bolton:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.05/C17

Topic: B.04. Synaptic Transmission

Support: NINDS/NIH R25 NS130964-02
NIMH125943
NIMH134515

Title: Electrophysiological investigation of Kirrel3 conditional knockout mice

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Abstract: Kirrel3, an autism and intellectual disability associated gene, encodes a neuronal cell adhesion molecule expressed in the dentate gyrus (DG) and GABAergic neurons of the CA3 region in the hippocampus. The DG transmits excitatory signals via mossy fiber (MF) filopodia to the CA3 where a balance of excitatory and inhibitory inputs converge to maintain proper CA3 pyramidal neuron activity levels. Disruption of the balance of excitation and inhibition in area CA3 can impair circuit and associated cognitive function. Previous studies indicated that Kirrel3 germline knockout (KO) mice have a specific reduction of DG-to-GABAergic interneuron MF filopodia synapses that, in turn, provide feed-forward inhibition to CA3 neurons. As a result, CA3 neurons in Kirrel3 KO mice receive a significantly reduced GABAergic synaptic response relative to their glutamatergic synaptic events originating from otherwise intact MF synapses.

These effects were determined by evaluating evoked excitatory post-synaptic currents (eEPSCs) and inhibitory post-synaptic currents (eIPSCs) in whole-cell voltage-clamped CA3 pyramidal neurons after direct electrical stimulation of MFs at the border of the DG hilus and stratum lucidum in area CA3 and calculating the ratio of excitation to inhibition (E/I ratio). Thus, Kirrel3 is necessary for normal hippocampal activity. Here, we set out to elucidate in precisely which neurons and at what developmental time points Kirrel3 participates in synapse formation in order to gain insight to its molecular mechanism(s). Because Kirrel3 is a homophilic cell adhesion molecule and is expressed in both the presynaptic DG neuron and the postsynaptic GABA neuron, we hypothesize that loss of Kirrel3 from either cell type will phenocopy the germline KO. To test this, we obtained Kirrel3 Floxed mice and crossed them with GABAergic interneuron specific Cre mice to generate mice lacking Kirrel3 only from these GABA-expressing interneurons. GABA-specific Kirrel3 knockout mice have significantly fewer DG-to-GABA mossy fiber filopodia synapses compared to controls, similar to germline KO mice. Here, we will present our new whole-cell voltage-clamp electrophysiological analysis of excitation / inhibition balance in the DG-GABA-CA3 circuit of these animals. This study contributes to understanding the role of Kirrel3 in hippocampal synapse formation and circuit function.

Disclosures: J.R. Muehlberger: None. **O. Shennib:** None. **P.J. West:** None. **M.E. Williams:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.06/C18

Topic: B.04. Synaptic Transmission

Title: Inhibitory ATLAS, a recombinant protein for anterograde tracing of inhibitory circuits

Authors: *H. SOHN[1,2], H. HUANG[1,2], J. RIVERA[2], D. B. ARNOLD[2];
[1]Neurosci. Grad. Program, [2]Dept. of Biol. Sciences, Dornsife Col., USC, Los Angeles, CA

Abstract: Previously, we developed ATLAS (Anterograde Transsynaptic Label based on Antibody-like Sensors), a recombinant protein that can mediate anterograde transsynaptic tracing from genetically determined neurons that is nontoxic, exclusively anterograde, and monosynaptic. A critical component of ATLAS is the AMPA.FingR, an antibody-like protein that binds at high affinity and specificity to the extracellular domain of the AMPA receptor, GluA1. ATLAS releases a peptide payload containing AMPA.FingR and Cre into the synaptic cleft, which binds to GluA1 at the postsynaptic membrane and is subsequently endocytosed. Thus, ATLAS can only mediate transsynaptic tracing from excitatory neurons. To generate a tracer capable of transsynaptic labeling from inhibitory neurons, we switched the AMPA.FingR for a nanobody that binds specifically to the alpha1 subunit of the GABAA receptor. In addition, the Synaptotagmin 1 nanobody[1], which targets ATLAS to synaptic vesicles, was switched for a minibinder against a pan-Synapsin epitope, PanSyn_BCMB, a minibinder generated in the Arnold

lab with Bindcraft[2]. Note that the lack of Synaptotagmin 1 at inhibitory synapses precludes using the Synaptotagmin 1 nanobody for synaptic vesicle targeting in inhibitory neurons. This inhibitory ATLAS retains the essential properties of the original system: monosynaptic, anterograde, genetic targeting, and activity dependence. In conclusion, by swapping out antibody-like proteins that bind to different targets from those used in our original tracer, we have created a version of ATLAS that can mediate anterograde transsynaptic tracing from inhibitory neurons. **Bibliography** 1. Queiroz Zetune Villa Real, K. *et al.* A Versatile Synaptotagmin-1 Nanobody Provides Perturbation-Free Live Synaptic Imaging And Low Linkage-Error in Super-Resolution Microscopy. *Small Methods* **7**, e2300218 (2023). 2. Pacesa, M. *et al.* BindCraft: one-shot design of functional protein binders. *bioRxiv* (2024) doi:10.1101/2024.09.30.615802.

Disclosures: H. Sohn: None. **H. Huang:** None. **J. Rivera:** None. **D.B. Arnold:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.07/C19

Topic: B.04. Synaptic Transmission

Support: R37MH080046

Title: Mcell-based computational modeling reveals how synaptic nanoarchitecture tunes NMDAR-mediated signaling

Authors: *R. LAGHAEI[1], M. ANDERSON[2], P. A. DHARMASRI[3], M. DAMENTI[3], S. R. METZBOWER[5], T. A. BLANPIED[4], A. D. LEVY[6];
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Abstract: Synaptic NMDA receptor (NMDAR) activation critically depends on the precise nanoscale organization of presynaptic release sites and postsynaptic receptors. In this work we investigated whether NMDA receptor (NMDAR) nanodomains align with presynaptic release machinery and how this affects receptor activation. Prior studies focused on AMPA receptors, this study shifts focus to NMDARs, which have slower kinetics and more complex subunit-specific signaling roles. We modeled the magnitude and time course of NMDAR activation in response to glutamate release using MCell simulation tool which enables detailed diffusion-reaction modeling using biologically realistic geometries. We used experimental data from super-resolved synapses from DNA-PAINT microscopy to constrain the spatial arrangement of biomolecules and synaptic architecture in our MCell models. We obtained 2D spatial maps of GluN2A and GluN2B, Munc13-1 (marking vesicle release sites) and PSD-95 (postsynaptic

scaffolding). We defined release site positions using Munc13-1 NC peaks, GluN2 subunit positions using their localizations. The synaptic boundaries were defined by merging the outlines of GluN2s, Munc13-1, and PSD-95. Release sites were placed at the centroids of Munc13-1 nanoclusters. NMDARs were distributed using the actual GluN2A/B localization data. We simulated glutamate release and receptor activation. We modeled the NMDAR kinetic schemes to predict the opening rates of each receptor in response to glutamate release based on the kinetic schemes derived from Santucci et al. The same simulations were run with randomized receptor and/or release site distributions, to assess the impact of spatial arrangement.
We found that while most NMDAR nanodomains were not directly aligned with vesicle release sites, those that are aligned across the synaptic cleft with the release sites (nanocolumns) exhibited significantly enhanced receptor open probability. Simulations revealed that even modest nanoscale offsets between release and receptor sites dramatically affected activation, particularly for GluN2B-containing receptors due to their slower glutamate binding kinetics. Randomizing the spatial organization of receptors or release sites diminished activation, highlighting the functional importance of native synaptic geometry. These findings demonstrate that synaptic nanoarchitecture is not only structurally diverse but also functionally specialized to enhance NMDAR signaling.
This work uncovers a novel principle of NMDAR regulation, indicating that synaptic function is shaped by the assembly and precise trans-synaptic alignment of multiprotein nanodomains.

Disclosures: R. Laghaei: None. **M. Anderson:** None. **P.A. Dharmasri:** None. **M. Damenti:** None. **S.R. Metzbower:** None. **T.A. Blanpied:** None. **A.D. Levy:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.08/C20

Topic: B.04. Synaptic Transmission

Support: NIH Grant 5F31NS129086

Title: Elucidating a role for presynaptic cell-adhesion molecule, PTPσ in coordinating the transsynaptic nano-alignment of postsynaptic NMDA receptors with presynaptic sites of vesicle release.

Authors: *E. DEMARCO[1,2], A. D. LEVY[2], T. A. BLANPIED[2];
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Abstract: Protein organization at individual synapses tunes synaptic strength. Postsynaptic receptors as well as presynaptic release-relevant proteins concentrate into sub-synaptic regions of high local density and can be aligned across the synapse, maximizing receptor activation following vesicle fusion. Our lab recently demonstrated that the optimal positioning of AMPA

receptors (AMPARs) is coordinated by the postsynaptic cell adhesion molecule (CAM) LRRTM2, as the acute disruption of its cleft interactions dispersed AMPARs from sites of evoked release and reduced EPSCs. This suggests a novel role for synaptic CAMs in controlling synapse strength through the regulation of nanostructure, and raises the question of whether NMDA receptors (NMDARs) are coordinated via a similar mechanism. An attractive model is that *presynaptic* CAMs linked to active zone (AZ) release machinery may convey structural information trans-synaptically to organize postsynaptic NMDARs. One candidate family for this are the LAR-RPTPs, which interact directly with the AZ protein Liprin-α and have several potential PSD-resident binding partners. Among the family members, PTPσ (*PTPRS*) and its trans-interactors have been associated with NMDAR dysfunction, well positioning it as a presynaptic CAM that may coordinate trans-synaptic alignment and coordinate the positioning of NMDARs. Here, we investigate the cellular expression and subsynaptic organization of endogenous PTPσ in established hippocampal neurons. Using confocal and DNA-Exchange PAINT imaging, we find that PTPσ is synaptically localized and expressed at 75% of excitatory synapses, while being at only 35% of inhibitory synapses. We assess the nanoscale localization of PTPσ to vesicle release sites, as well as their subsynaptic localization with respect to postsynaptic NMDARs. Lastly, we test whether PTPσ is critical for presynaptic nanostructure or the localization of NMDARs near subsets of vesicle release sites. Altogether, elucidating the key players involved in coordinating nanostructure and maintaining trans-synaptic positioning of NMDARs to sites of presynaptic release will provide candidate molecules and new concepts for regulation of synaptic strength and plasticity.

Disclosures: E. DeMarco: None. **A.D. Levy:** None. **T.A. Blanpied:** None.

Poster

PSTR375: Transsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.09/C21

Topic: B.04. Synaptic Transmission

Support: F31NS141417
R01NS110383

Title: Amyloid-beta oligomer (Aβo)-induced fragmentation of synaptic nanostructure

Authors: *H. J. RAMSAY, M. J. KENNEDY;
Pharmacol., Univ. of Colorado, Anschutz Med. Campus, Aurora, CO

Abstract: Many postsynaptic proteins, including AMPA-type glutamate receptors (AMPARs), organize into nanometer scale subsynaptic domains (SSDs) that are found in close proximity to putative presynaptic neurotransmitter release sites. While precise localization of synaptic proteins plays a key role in synaptic function, it is currently unclear whether any brain disease-related synaptic perturbations could be a result of disruptions to synaptic nanoarchitecture.

Alzheimer's Disease (AD) is associated with accumulation of soluble Amyloid-Beta oligomers (Aβo) at a subset of excitatory synapses, where they disrupt plasticity within minutes and trigger synapse elimination within days. Here we interrogated whether acute (15min-3h) exposure to Aβo had any effect on synaptic nanoarchitecture. We observed rapid (within 15 min) increase in the number of PSD95 and AMPAR SSDs. This nanoscale "fragmentation" of postsynaptic receptor and scaffold proteins was observed at Aβo-bound spines but not neighboring spines on the same neuron with little or no detectable Aβo. Longitudinal live imaging before and during Aβo exposure revealed no significant changes in the overall spine levels of PSD95 or GluA1, supporting a model where nanostructural changes arise from disruption of existing synaptic proteins, rather than integration of additional scaffold or receptor SSDs. Intriguingly, pharmacological antagonism of NMDARs led to a similarly rapid postsynaptic fragmentation that occluded the effect of Aβo, suggesting Aβo-induced fragmentation is mediated through alterations to NMDAR signaling. Finally, we could prevent Aβo-induced synapse fragmentation by antibody-crosslinking GluA1-containing AMPARs prior to Aβo application. This will allow us to test if synapse fragmentation is a required step in Aβo-induced synapse elimination. Experimental model / rigor statement: In this confirmatory work, cultured neurons from male/female P0-P1 Sprague-Dawley rat pups were pooled and used for all experiments. Biological sex differences were not assessed. We ensured scientific rigor using appropriate controls, biological/technical replicates with sufficient sample size, and blinding when possible.

Disclosures: H.J. Ramsay: None. **M.J. Kennedy:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.01/C22

Topic: B.05. Synaptic Plasticity

Support: CIHR FRN180241

Title: Prenatal exposure to vaporized delta-9-tetrahydrocannabinol reduces synaptic potentiation in the hippocampus

Authors: *R. PRZY[1], C. ACOSTA[2], J. D. THOMAS[3], B. R. CHRISTIE[1,3,4];
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Abstract: Cannabis is one of the mostly commonly used substances during pregnancy, where it is often used for therapeutic reasons, despite limited understanding of how exposure to delta-9-tetrahydrocannabinol (THC) affects fetal neurodevelopment. The high expression of cannabinoid type-1 receptors (CB1R) in the hippocampus makes this memory-related brain region a key target in exploring the effects of developmental THC exposure. The objective of this study is to

investigate whether prenatal exposure to vaporized THC impacts synaptic plasticity in the rodent hippocampus. Pregnant Sprague-Dawley rats were randomized to receive passive exposure to THC or vehicle (control) vapor daily from gestational days 5 to 20. Male and female offspring were aged to young adulthood (~ postnatal day 55) and their brains were extracted for *ex vivo* field electrophysiology in the medial perforant pathway of the hippocampus. A maximum of two pups/sex/dam were used to control for litter effects. Long-term potentiation (LTP) and long-term depression (LTD) were assessed using theta-burst (TBS) and low-frequency (LFS) conditioning stimuli, respectively. Data analysis was completed blinded to experimental condition and sex. Preliminary findings indicate that prenatal THC-exposed offspring exhibit reduced long-term potentiation (LTP), with no effect on long-term depression (LTD) in the medial perforant pathway. These results highlight the potential for prenatal exposure to vaporized THC to induce long-term synaptic deficits in the hippocampus that may adversely impact learning and memory. Ongoing experiments will evaluate whether prenatal THC exposure alters the role of CB1R signaling in LTP and LTD.

Disclosures: R. Przy: None. **C. Acosta:** None. **J.D. Thomas:** None. **B.R. Christie:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.02/C23

Topic: B.05. Synaptic Plasticity

Support: P50 DA044118

Title: Adolescent THC diminishes effects of estrogen on excitatory transmission in adult hippocampus

Authors: *A. A. LE[1], J. C. LAUTERBORN[2], D. PIOMELLI[3], G. LYNCH[4], C. M. GALL[5]; [2]Dept Anat. & Neurobio., [1]Univ. of California Irvine, Irvine, CA; [3]Univ. California Irvine, Irvine, CA; [4]Univ. California, Irvine, Irvine, CA; [5]Dept. of Anat. and Neurobio., Univ. of California at Irvine, Irvine, CA

Abstract: There is evidence that cannabis use during adolescence leads to cognitive impairments in young adulthood but little is known about effects of early-life cannabis exposure on synaptic operations that are critical for encoding and organizing information. We previously reported that in rats and mice exposure to Δ9-tetrahydrocannabinol (THC) daily for 2 weeks during adolescence leads to a marked impairment in hippocampal field CA1, Schaffer-commissural (S-C) long-term potentiation (LTP) in females, but not males, assessed in adulthood. Moreover, previous studies show that S-C LTP depends on estrogen, and synaptic estrogen receptors, in females only. Together, these results suggest that early THC exposure may disrupt synaptic estrogen action. Initial tests of this possibility demonstrated that in females treated with THC during adolescence (ado-THC: 5 mg/kg, daily from postnatal day 30-43) the normal

enhancement of S-C transmission by β-estradiol (E2) infusion is absent. The present studies used field recordings from hippocampal slices to test if ado-THC disrupts estrogen influences on S-C evoked responses in both male and female mice, and the estrogen receptors involved. Females were tested outside proestrus. Results show that E2 infusion increased S-C field excitatory synaptic potential (fEPSP) slopes in slices from adult (2-4 mo old) male and female mice given vehicle during adolescence. In both sexes, effects of E2 infusion on S-C fEPSPs were significantly smaller in mice given ado-THC. Similarly, in both sexes, infusion of the estrogen receptor β (ERβ) agonist DPN increased the magnitude of S-C fEPSPs in slices from vehicle-treated mice and this response was significantly reduced in ado-THC mice. These results indicate that changes in ERβ function contribute to the loss of estrogen responsivity in both males and females exposed to THC during adolescence. Current studies are testing if changes in synaptic estrogen receptor levels contribute to these effects.

Disclosures: A.A. Le: None. **J.C. Lauterborn:** None. **D. Piomelli:** None. **G. Lynch:** None. **C.M. Gall:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.03/C24

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 206800

Title: Developmental induction of synaptic long-term depression in the hippocampus: Using chimeric GluN2 subunits to establish domain-specific contingencies of pathological plasticity

Authors: *L. C. MCAULIFFE, T. M. SAED, E. B. WRIGHT, T. C. DUMAS;
George Mason Univ., Fairfax, VA

Abstract: Presynaptic glutamate release and concomitant postsynaptic depolarization activates N-methyl-D-aspartate receptors (NMDARs) to drive long lasting changes in synaptic efficacy. Activity-dependent weakening of synaptic strength (long-term depression, LTD), which can be induced via ionotropic or nonionotropic NMDAR signaling, is more easily generated at immature synapses in juvenile rodents than in mature animals. Interestingly, hippocampal NMDAR composition changes in parallel with the developmental shift in LTD induction, such that as GluN2A subunit-containing NMDARs increase, LTD becomes harder to induce. However, it is not known if NMDAR composition is causally related to the developmental reduction in LTD and, if so, whether the effect is driven by ionotropic or nonionotropic signaling. Since GluN2B expression is constitutive in postnatal development, we hypothesized that heightened GluN2A-type ionotropic or nonionotropic signaling is responsible for the decrease in LTD induction observed with increasing postnatal age. We tested this hypothesis by recording electrically evoked synaptic field responses in acutely prepared hippocampal slices

from two transgenic mouse lines expressing chimeric GluN2 subunits (GluN2A-B^{CTD}: GluN2B intracellular domain, or CTD, fused to GluN2A extracellular domains; GluN2B-A^{CTD}: GluN2A CTD fused to GluNB extracellular domains), which permit experimental separation of ionotropic and nonionotropic signaling. Low frequency stimulation (LFS, 1 Hz for 15 minutes) did not induce LTD in slices prepared at postnatal days (P) 22-24 from any experimental group, though it did produce LTD in slices from all experimental groups at P17-19. LTD was accompanied by an increase in paired-pulse facilitation in slices from GluN2A-B^{CTD} transgenic mice and wildtype controls, but not from GluN2B-A^{CTD} mice. These data show that enhancement of GluN2A-type CTD signaling at immature synapses promotes an inhibitory effect on LTD induction and suggest the GluN2B CTD regulates presynaptic function either retrogradely, possibly via PSD95/neuroligin/neurexin, or by presynaptic NMDARs on Schaffer collateral terminals. Synaptic response parameters will be analyzed via a two-way analysis of variance (ANOVA) evaluating age and genotype differences followed by post-hoc analyses of sex effects. Results of the study will provide a further understanding of the molecular underpinnings of LTD on the level of individual NMDA receptor subunit domains, allowing for more specific pharmacological targets for the treatment of various cognitive, mood, and neurodegenerative disorders.

Disclosures: L.C. McAuliffe: None. **T.M. Saed:** None. **E.B. Wright:** None. **T.C. Dumas:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.04/C25

Topic: B.05. Synaptic Plasticity

Support: R01 DA 011289

Title: Somatodendritic release of cholecystokinin (CCK) modulates synaptic inputs to VTA dopamine neurons

Authors: *S. SIANATI[1], J. A. KAUER[2];
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Abstract: Neuropeptides are found in nearly every brain neuron, and can modulate behaviors by regulating neuronal excitability, synaptic transmission, and plasticity. In contrast to the canonical view of neuropeptide release from nerve terminals, we recently reported the somatodendritic release of CCK from ventral tegmental area (VTA) dopamine (DA) neurons. Release of CCK occurs during modest sustained depolarization of VTA DA cells, and by activating CCK2Rs, potentiates synaptic transmission from GABAergic afferents (Martinez Damonte et al., 2023; doi: 10.1016/j.biopsych.2022.06.011). Somatodendritic neuropeptide release remains understudied; here we have further investigated how CCK release modulates synaptic inputs to VTA DA neurons, and whether kappa opioid receptor (KOR) signaling affects these

processes.Using whole-cell patch-clamp recordings, we found that in addition to eliciting LTP of GABAergic synapses, somatodendritic CCK release drives LTP of excitatory synapses on VTA DA neurons. To determine whether CCK potentiates $GABA_A$ synapses via pre- or postsynaptic mechanisms, we blocked G-protein signaling in DA cells with intracellular GDPβS. Postsynaptically-delivered GDPβS did not prevent CCK-induced LTP as expected if CCK acts via postsynaptic GPCRs. Instead, our results suggest that CCK acts on presynaptic GABAergic terminals in the VTA.KORs are highly expressed in the VTA and multiple dynorphin afferents innervate the region. Previous work in VTA DA neurons showed that KOR activation disrupts D2 receptor IPSCs, likely by preventing somatodendritic release of DA (Ford et al., 2007: doi: 10.1152/jn.00963.2006). We hypothesized that KOR signaling might also inhibit CCK release or signaling in the same cells. Indeed, pharmacological activation of KOR prevented CCK-induced LTP of inhibitory inputs. However, this inhibition was not prevented by blocking of G-protein signaling in the postsynaptic DA cells. In contrast to postsynaptic KOR effects on dopamine IPSCs, these results support a presynaptic action of KORs that opposes CCK2R signaling,We are now performing simultaneous paired recordings from adjacent VTA DA neurons. This approach allows us to determine whether LTP at $GABA_A$ synapses can spread to neighboring cells following CCK release from a nearby neuron. Overall, our findings suggest that CCK decreases the excitability of DA cells, both by potentiating inhibitory and suppressing excitatory synaptic transmission through predominantly presynaptic mechanisms. Furthermore, KORs inhibit CCK-induced LTP, and thus KOR activation is expected to oppose the effects of somatodendritic CCK release on dopamine neurons.

Disclosures: S. Sianati: None. **J.A. Kauer:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.05/C26

Topic: B.05. Synaptic Plasticity

Title: Dopamine-mediated dnmt3a isoform dynamics drive striatal synaptic plasticity: implications for mood disorders

Authors: *S. E. REID[1], L. LAVERY[2];
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Abstract: Major depressive disorder (MDD) profoundly impacts society, characterized by dysfunctional reward learning and altered synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), in regions like the striatum. Epigenetic factors, particularly DNA methyltransferase Dnmt3a, are critical for synaptic plasticity and cognition. While Dnmt3a's role in LTP is known, the distinct isoform-specific involvement of Dnmt3a in LTD, its downstream epigenetic targets, and potential isoform collaboration in healthy vs. maladaptive plasticity remain poorly understood. This study investigates the distinct roles of

Dnmt3a isoforms and immediate early gene (IEG) expression during chemically induced LTP and LTD in primary mouse striatal neuronal cultures and their contribution to maladaptive plasticity using *in vivo* mouse models of depression. To induce LTP and LTD, we utilized established chemical protocols in primary striatal neuron cultures (derived from newborn C57BL/6J mice, both sexes). Gene expression of Dnmt3a1, Dnmt3a2, and IEGs (*e.g.,* FosB, cFos, Egr2, Arc) was quantified via reverse transcription quantitative PCR (RT-qPCR). Our preliminary data demonstrate that chemical LTP induction via dopamine (DA) agonist (SKF38393, 10µM, n=3 wells per group, three studies) significantly increased expression of Dnmt3a2 and all tested IEGs. Notably, Dnmt3a1 expression remained unchanged under these LTP-inducing conditions, which was expected. This DA-agonist-mediated potentiation of gene expression was abolished when co-treated with a DA antagonist (SCH23390, 10µM), with expression levels returning to control values. These findings indicate a specific role for Dnmt3a2, but not Dnmt3a1, in DA-modulated striatal LTP-related gene expression. We hypothesize that Dnmt3a1 plays a more critical role in LTD than LTP, given the distinct transcriptional demands during LTD. To further elucidate the *in vivo* relevance, we have developed genetically modified mice enabling conditional specific knockout of either Dnmt3a1 or Dnmt3a2. These models will be subjected to the chronic social defeat stress (CSDS) paradigm to examine the isoform-specific contribution of Dnmt3a to maladaptive synaptic plasticity and depressive-like behaviors. Rigor is ensured through controlled culture conditions, randomized animal assignment, blinded analyses, and statistical analysis of multiple biological/technical replicates. This research aims to identify novel epigenetic mechanisms underlying striatal synaptic dysfunction and offers isoform-specific therapeutic targets for MDD.

Disclosures: S.E. Reid: None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.06/C27

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS111986

Title: Differential effects of CB1 receptor antagonism on hippocampal long-term depression during aging

Authors: F. LEMTIRI-CHLIEH[1], *E. S. LEVINE[2];
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Abstract: The temporally and spatially restricted release of endogenous cannabinoids in the brain can mediate or modulate synaptic plasticity, including long-term potentiation and long-term depression (LTD), in a synapse-specific manner. This is especially relevant in the hippocampus, a key structure involved in learning and memory. Brain aging is associated with a

decline in cognitive function that parallels changes in synaptic plasticity. Endocannabinoid signaling also undergoes marked changes over the lifespan, with decreased activity in middle-aged animals and variable outcomes reported in aged animals. The goal of the present studies was to explore the role of altered endocannabinoid signaling in age-dependent changes in hippocampal LTD. We examined the role of endocannabinoid signaling in LTD by recording field excitatory postsynaptic potentials (fEPSPs) in the CA1 stratum radiatum in hippocampal slices from adolescent (15 - 40 days), mature (3 - 4 months), and aged (22 - 24 months) mice. LTD was induced by a 10-minute exposure to the metabotropic glutamate receptor agonist (S)-3,5-dihydroxyphenylglycine (DHPG). In adolescent and mature animals, significant LTD (30 - 40% decrease from baseline) could be induced by 10 min of exposure to DHPG and lasted at least sixty minutes. This form of LTD was dependent on mGluR activation because it was completely blocked by combined exposure to the mGluR1 antagonists MPEP and CPCCOEt. In adolescent animals, the magnitude of LTD was significantly reduced by blocking cannabinoid receptor activation with the CB1 receptor antagonist NESS-0327. In contrast, the magnitude of LTD in mature animals was generally unaffected by blocking cannabinoid receptor activation. In aged mice, LTD was not consistently evoked, and the magnitude of LTD when present was significantly lower than in juvenile and mature animals. Interestingly, however, the magnitude of LTD in aged animals was increased by blocking cannabinoid receptor activation with NESS-0327. These results indicate that the magnitude and prevalence of DHPG-induced LTD are age-dependent, as are the effects of CB1 receptor blockade. This suggests that endocannabinoid signaling plays differential roles in synaptic plasticity across the lifespan. Understanding the age-dependent roles of endocannabinoid signaling will be critical in unraveling their contributions to synaptic plasticity, learning, and memory during early development and aging.

Disclosures: F. Lemtiri-Chlieh: None. **E.S. Levine:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.07/C28

Topic: B.05. Synaptic Plasticity

Support: NIA Grant AG076835
Hewitt Foundation Fellowship

Title: Unlocking memory potential-Targeting ACC1 facilitates synaptic plasticity and long-term memory formation in the adult and aging mouse brain

Authors: *M. DEY[1], F. G. GARCIA[4], E. A. KRAMAR[5], J. PICONE[2], M. A. WOOD[3]; [1]Neurobio. and Behavior, [2]Anat. and Neurobio., [3]Neurobiol & Behavior, Univ. of California Irvine, Irvine, CA; [4]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA; [5]Neurobio. and Behavior, Univerisity of California, Irvine, Irvine, CA

Abstract: Epigenetics involves both inherited and non-inherited mechanisms that control gene expression by altering chromatin structure, without changing the underlying DNA sequence. Recent studies have highlighted a significant connection between metabolic activity and epigenetic changes, showing that metabolic intermediates are key players in modifications like DNA/RNA methylation, histone acetylation etc. Despite these advances, the precise relationship between metabolism and epigenetic regulation in the context of learning and memory remains unclear. This research investigates the hypothesis that acetyl-CoA is essential for histone acetylation and the activation of genes involved in long-term memory (LTM) formation. Using a combination of molecular, electrophysiological, and behavioral techniques in mouse models, the study aims to explore how metabolic processes affect epigenetic mechanisms during memory formation. Early findings suggest that inhibiting acetyl-CoA carboxylase 1 (ACC1) elevates both acetyl-CoA levels and long-term potentiation (LTP) in the hippocampus, which is associated with improved memory performance. The research also seeks to clarify ACC1's role in regulating acetyl-CoA concentrations, histone acetylation, and the expression of genes linked to memory. The anticipated outcomes may provide deeper insight into the regulation of memory and age-related cognitive decline, and potentially inform new treatments for neurodegenerative diseases like Alzheimer's.

Disclosures: M. Dey: A. Employment/Salary (full or part-time): 1. Department of Neurobiology and Behavior, University of California Irvine, Irvine, CA, 92697, USA. **F.G. Garcia:** A. Employment/Salary (full or part-time): 1.Department of Neurobiology and Behavior, University of California Irvine, Irvine, CA, 92697, USA. **E.A. Kramar:** A. Employment/Salary (full or part-time): 1. Department of Neurobiology and Behavior, University of California Irvine, Irvine, CA, 92697, USA. **J. Picone:** A. Employment/Salary (full or part-time): Department of Anatomy and Neurobiology, University of California Irvine, Irvine, CA, 92697, USA. **M.A. Wood:** A. Employment/Salary (full or part-time): 1. Department of Neurobiology and Behavior, University of California Irvine, Irvine, CA, 92697, USA, 2. Center for the Neurobiology of Learning and Memory (CNLM), University of California, Irvine, Irvine, CA, 92697, USA, 3. Institute for Memory Impairments and Neurological Disorders (UCI MIND), University of California, Irvine, Irvine, CA, 92697, USA.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.08/C29

Topic: B.05. Synaptic Plasticity

Support: NIH R01AA025718
Fondecyt-1221080
PhD ANID fellowship-21202521

Title: Early CP-AMPAR dysregulation contributes to impaired LTD in D1R+ medium spiny neurons of the nucleus accumbens in a transgenic Alzheimer's disease mouse model

Authors: *N. RIFFO-LEPE[1,2], J. GONZÁLEZ-SANMIGUEL[2], L. ARMIJO WEINGART[2], P. SAAVEDRA SIEYES[2], I. MEZA VARGAS[2], H. ZAMBRANO[2], A. RIQUELME[2], L. S. SAN MARTIN[2], G. RAMOS FLORES[2], L. G. AGUAYO[2];
[2]Physiol. Dept., [1]Univ. of Concepción, Concepción, Chile

Abstract: Alzheimer's disease (AD) is classically defined by progressive cognitive decline, but early non-cognitive symptoms such as apathy and compulsive behavior suggest that dysfunction in reward-related circuits may precede memory impairment. The nucleus accumbens (nAc), a mesolimbic central hub, has been proposed as an early target of AD pathology. We used APPswe/PS1E9d (2xTg) mice crossed with Drd1a-TdTomato mice to label D1 receptor-expressing medium spiny neurons (D1+ MSNs). At 6 months, we observed intracellular amyloid-beta (Aβ) accumulation in both D1+ and D1− MSNs without extracellular plaques. Whole-cell voltage-clamp recordings showed impaired LTD induction selectively in 2xTg D1+ MSNs after high-frequency stimulation or mGluR1/5 activation. Although mGluR1/5-LTD normally promotes synaptic removal of calcium-permeable AMPA receptors (CP-AMPARs), DHPG failed to induce LTD in 2xTg D1+ MSNs. This was accompanied by increased rectification index, NASPM sensitivity, and GluA1 expression, suggesting impaired CP-AMPAR clearance. GCaMP6s-associated imaging confirmed elevated AMPAR-mediated calcium responses in 2xTg mice. Consistently, protein analysis of nAc tissue revealed significant increases in postsynaptic markers PSD95, GluA1, and GluA2, while the presynaptic vesicle marker SV2 remained unchanged. Moreover, paired-pulse ratio analysis revealed no differences in presynaptic neurotransmitter release between WT and 2xTg animals, supporting the idea of selective postsynaptic remodeling. Anxiety-like behavior in the elevated plus maze and social interaction were unaltered. However, conditioned place preference (CPP) using chocolate revealed increased consumption in 2xTg mice. Notably, photometric recordings using DLight1.1 in acutely prepared brain slices revealed reduced electrically evoked dopamine release in the nAc of 2xTg mice, indicating impaired mesolimbic dopaminergic responsiveness that may underlie their increased reward-seeking behavior. These findings show that D1+ MSNs in the nAc undergo early synaptic and neuromodulatory alterations prior to plaque formation, potentially contributing to non-cognitive symptoms of AD. This work supports the idea that mesolimbic circuit dysfunction is an early and functionally relevant pathological feature of the disease.

Disclosures: N. Riffo-Lepe: None. **J. González-Sanmiguel:** None. **L. Armijo Weingart:** None. **P. Saavedra Sieyes:** None. **I. Meza Vargas:** None. **H. Zambrano:** None. **A. Riquelme:** None. **L.S. San Martin:** None. **G. Ramos Flores:** None. **L.G. Aguayo:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.09/C30

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS111986

Title: Differential role of BDNF-TrkB signaling in hippocampal long-term potentiation during aging

Authors: *J. RAINGO[1], E. S. LEVINE[2];
[2]Neurosci., [1]Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Understanding how aging impacts hippocampal synaptic plasticity could aid in developing therapeutics to ameliorate cognitive and memory decline in elderly patients. Among many physiological changes, reduced brain-derived neurotrophic factor (BDNF) signaling is particularly detrimental to synaptic plasticity, memory, and cognitive processes in aged individuals. Our goal here is to investigate age-related changes in BDNF-dependent synaptic plasticity in the CA1-CA3 hippocampal circuit of juvenile, adult, and aged mice. We examined the role of BDNF in various forms of long-term potentiation (LTP) by recording field excitatory postsynaptic potentials (fEPSPs) in the CA1 region while stimulating the Schaffer collateral fibers from the CA3 region in hippocampal slices obtained from mice aged 1-3, 9-12, and 22-24 months. We first compared LTP evoked by a 30-minute bath application of the TrkB agonist 7,8-dihydroxyflavone (DHF) in juvenile and aged mouse hippocampi. In both age groups, DHF induced sustained potentiation of fEPSPs lasting for 60 minutes after washout (DHF 2 μM: juvenile mice: 196.0 ± 30.3% of baseline; aged mice: 184.3 ± 16.9% of baseline; $n = 5$ per group). This effect was dose-dependent and completely blocked by the TrkB antagonist K252a (200 nM). This indicates that TrkB receptors and downstream signaling remain available to support synaptic plasticity in juvenile and aged hippocampal circuits. Next, we explored the effect of aging on LTP induced by various electrical stimulation protocols. Both theta burst stimulation (TBS; 10 bursts, 5 spikes/burst) and 200 Hz tetanic stimulation (10 bursts, 40 spikes/burst at 0.2 Hz) induced similar LTP (~160% of baseline) across all age groups. However, the role of BDNF signaling showed age-related differences: For TBS, the K252a-sensitive component of LTP was greatest in juvenile mice and diminished with age. Using weaker stimulation protocols (1-3 bursts), we observed an age-related reduction in the magnitude of LTP. Under these conditions, inhibition of TrkB completely blocked LTP in juvenile mice but had no effect in aged mice. In contrast, 200 Hz-induced LTP was sensitive to TrkB inhibition only in the aged group. Our findings indicate that BDNF signaling remains functional and can contribute to synaptic plasticity across different age groups. However, the role of endogenous BDNF in LTP at different ages varies depending on the induction paradigm, suggesting that the underlying mechanisms of synaptic plasticity change with aging.

Disclosures: J. Raingo: None. **E.S. Levine:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.10/C31

Topic: B.05. Synaptic Plasticity

Title: Prefrontal cortical cholinergic signaling in models ofα-synucleinopathy

Authors: *J. REINHARDT[1], N. E. CHAMBERS[2], I. GALLARDO[1], M. S. MOEHLE[3];
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Abstract: Progressive loss of basal forebrain cholinergic projection neurons is predictive of cognitive deficits in dementia with Lewy bodies and Parkinson's Disease dementia, and aggregates of α-synuclein robustly accumulates in the basal forebrain of patients. Aggregates of α-synuclein have been shown to impair synaptic transmission by disrupting vesicle recycling, SNARE complex function, and receptor trafficking, leading to reduced glutamate and dopamine release, excitotoxicity, and mitochondrial dysfunction. However, it remains unclear how a-synuclein aggregation impairs cholinergic neurons in the basal forebrain, acetylcholine release from basal forebrain neurons and postsynaptic cholinergic signaling. To address this, we use the preformed fibril model of α -synucleinopathies in rodents to induce robust α-synuclein aggregation like that seen in patients. We observe cholinergic fiber loss and accumulation of α-synuclein in basal forebrain projection neurons. Using whole-cell patch clamping electrophysiology we examine cholinergic dependent plasticity in the prefrontal cortex as well as cholinergic signaling in layer V pyramidal neurons. We observe increased long-term depression in layer V pyramidal neurons harboring Lewy like pathology. Additionally, we observe changes in cholinergic signaling and receptor expression. Taking together our data suggests α-synuclein aggregation leads to an anatomical rearrangement of basal forebrain cholinergic neurons that leads to altered plasticity and signaling in the prefrontal cortex, which may underlie executive function deficits in these disorders. We predict that, by selectively modulating muscarinic signaling in the prefrontal cortex, we may be able to normalize plasticity deficits and thereby normalize executive function, and that this may be a novel therapeutic approach for α-synuclein linked dementias.

Disclosures: J. Reinhardt: None. **N.E. Chambers:** None. **I. Gallardo:** None. **M.S. Moehle:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.11/C32

Topic: B.05. Synaptic Plasticity

Title: Striatal and Hippocampal Long-Term Potentiation in a Kindling Mouse Model of Temporal Lobe Epilepsy

Authors: *C. DAUT[1], F. TESCAROLLO[2], S. C.-Y. CHEN[3], H. SUN[4];
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Abstract: The striatum, a portion of the basal ganglia that coordinates motor learning, is implicated in the pathogenesis of hippocampus-originated seizures in mesial temporal lobe epilepsy (mTLE). mTLE is characterized by recurring spontaneous seizures that can affect additional areas through the spreading of hypersynchronous activity, with known comorbidities of motor learning and memory decline. We aimed to examine whether excitatory and inhibitory synaptic marker levels are associated with cognitive deficits secondary to progressive seizure intensification, using our optogenetic kindling mouse model of mTLE (OpK) (n=14) to induce seizures. Intra-hippocampal kainic acid-injected mice (KA) (n=10) served as a model for chronic mTLE, with naïve mice (n=12) as controls. We saw that repeated seizure exposure resulted in an increased expression of $GABA_a$ with accompanying deficits in hippocampal and dorsolateral striatum-dependent spatial memory performance. KA and OpK mice showed respective 723% ($p<0.0001$) and 417% ($p=0.0001$) increases in $GABA_a$ expression compared to naïve mice in the hippocampus, with KA mice showing a greater increase in hippocampal $GABA_a$ compared to OpK mice ($p=0.0151$). KA and OpK mice showed respective 257% ($p<0.0001$) and 160% ($p=0.0002$) increases in striatal $GABA_a$ expression. No statistically significant changes were observed in vGlut1 for KA or OpK. Morris water maze (WMT) and novel object recognition (NORT) tests were conducted to investigate alterations in spatial learning and memory. OpK mice showed decreased WMT target crossings compared to pre-kindling trials ($p=0.001$, paired t-test). Compared to pre-KA injection trials, KA mice trended toward fewer crossings ($p=0.0564$, paired t-test). Memory deficits from NORT were inconclusive. Marked changes in $GABA_a$ expression without clear vGlut1 changes indicate that primary alterations in kindling and chronic epilepsy lie in the inhibitory synapses, suggesting a striatal projection neuron response of increased sensitivity to GABAergic inhibition. This is likely a curbing response to the overwhelming excitatory drive of seizure activity. In the KA model of chronic epilepsy, which causes hippocampal sclerosis, the need for increased sensitivity may be further driven by a loss of GABAergic neurons. Between active seizures, this increased inhibition sensitivity may produce a lower basal activity level, thus impairing normal functions of affected brain regions. The demonstrated reduced memory-associated behavior performance likely reflects depressed neural processing in the hippocampus and striatum as a result of acute $GABA_a$ synaptic changes, rather than chronic pathology.

Disclosures: C. Daut: None. **F. Tescarollo:** None. **S.C. Chen:** None. **H. Sun:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.12/C33

Topic: B.05. Synaptic Plasticity

Support: NSERC Grant RGPIN-2019-06104

Title: Optimizing adult brain slice viability with N-Methyl-D-Glucamine: implications for synaptic plasticity in ex vivo field electrophysiology recordings

Authors: *P. GOMES WELTER[1,2], J. HOFMEYER[2], F. MORADPOUR[2], J. BRAND[2], E. EYOLFSON[2], P. S. BROCARDO[1], B. R. CHRISTIE[2,1];
[1]Federal Univ. of Santa Catarina, Florianópolis, Brazil; [2]Univ. of Victoria, Victoria, BC, Canada

Abstract: Introduction: A major challenge in electrophysiological studies is investigating synaptic plasticity ex vivo in adult brain tissue, due to the poor viability of slices and difficulties in obtaining reliable recordings. As a result, most research focuses on juvenile animals, limiting our understanding of adult synaptic function. This study has been done to address this gap by optimizing brain slice preparation using an N-methyl-D-glucamine (NMDG)-based solution. Although this approach is commonly employed in whole-cell recordings, its effectiveness for field recordings and its impact on long-term potentiation (LTP) induction remain unclear. **Methods:** Male and female Sprague-Dawley rats aged postnatal day (PND) 73-81 were assigned to two experimental groups: artificial cerebrospinal fluid (aCSF) and NMDG. In the NMDG group, the NMDG-based solution was used during perfusion, slicing, and the initial recovery phase, while aCSF was subsequently used in the holding chamber and during recordings. In contrast, the aCSF group was maintained in aCSF throughout all stages, from perfusion to recording. Synaptic efficacy was evaluated by inducing long-term potentiation (LTP) using a Theta Burst Stimulation (TBS) protocol (4 trains, 5 bursts/train, 5 pulses at 100 Hz/burst, 200ms inter-burst interval, totaling 100 pulses). **Results:** While NMDG treatment led to better-preserved slices for whole-cell recordings, the amount of LTP was reduced compared to the aCSF group. **Conclusions:** Although the NMDG-based protocol enhanced tissue viability for whole-cell recordings, our preliminary data indicate that slice preparation in NMDG is associated with diminished LTP when compared to preparation in standard aCSF. This outcome may indicate that the solution used for ex vivo preparations can dramatically impact slice health and its capacity for sustaining LTP. Future studies will integrate this electrophysiology approach with protein analysis of AMPA/NMDA receptor ratios in tissue sections, and synaptic immunohistochemistry, to better elucidate the synaptic changes associated with different preparation solutions.

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Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.13/C34

Topic: B.05. Synaptic Plasticity

Support: NS074256
AG046929

Title: Impact of Somatostatin-Expressing Inhibitory Neuron Specific BACE1 Deletion in Synaptic Plasticity

Authors: *M. KANDEL[1], R. YAN[2];
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Abstract: BACE1, an enzyme involved in APP processing, is a prime therapeutic target for treating Alzheimer's disease. Inhibitory interneurons make up around 15-20% of the total neuron population in the cerebral cortex. It is generally understood that there are three distinct and non-overlapping interneuron classes in the mouse neocortex, namely, parvalbumin (PV) -expressing, 5-HT_{3A} receptor-expressing and somatostatin (Sst)-expressing interneuron classes. PV and Sst interneuron dysfunctions have been implicated in AD models. Here we prepared the conditional BACE1 deletion in Sst-expressing cells by crossing the BACE1 flox/flox mice with Sst-IRES-Cre mice, obtaining mice homozygous for BACE1 floxed allele and heterozygous for the Sst-IRES-Cre allele (BACE1 fl/fl;Sst_IRES-Cre +/WT, or Sst-BACE1-KO mice). We found that the field excitatory post synaptic potentials (fEPSPs) have been significantly inhibited in these Sst-BACE1-KO mice when compared with the control (BACE1 fl/fl mice). Fear Conditioning Behavior data showed a significant decrease in freezing time during time component (s) by Sst-BACE1-KO mouse when compared with the control (BACE1 fl/fl) on day 3rd Cue. No change was observed in open filed and y-maze behavior between the groups. EEG recording indicates no seizure activity in Sst-BACE1-KO mice when compared with control (BACE1 fl/fl mice). These data suggest the important role of inhibitory BACE1 in synaptic plasticity and function. Future directions include the measurement of intrinsic membrane properties, miniature inhibitory post-synaptic currents (IPSCs) and evoked IPSCs.

Disclosures: M. Kandel: None. **R. Yan:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.14/C35

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS102239

Title: Loss or block of Slack channels prevents long-term potentiation

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Abstract: Slack (also known as KCNT1, Slo2.2) is a sodium-activated potassium channel found throughout the brain. Gain-of-function Slack mutations lead to several disorders characterized by severe infantile seizures as well as profound and permanent intellectual disability. Knockout of the gene for Slack in mice disrupts cognitive behaviors in intact animals as well as synaptic plasticity recorded in brain slices. We have now tested the effect of loss of the Slack gene, as well as acute pharmacological block of the channel on chemical long-term potentiation (LTP)

measured in E17 primary hippocampal neurons. On in vitro day 14, neurons were stimulated with the NMDA receptor co-agonist D-serine for 30 minutes to induce chemical LTP, and then, after an additional 30 minutes in D-serine-free medium, fixed for confocal immunomicroscopy. In neurons from wild-type animals, the intensity of surface staining for the AMPA receptor subunit GluR1 in the neurites was significantly increased compared to that in neurons not exposed to D-serine, and this increase was blocked by pretreatment of the cells with the NMDA receptor inhibitor 2-amino-5-phosphonovaleric acid. In contrast to wild-type neurons, no increase in surface GluR1 could be detected in Slack knockout neurons in response to D-serine treatment, confirming the requirement of Slack channels for LTP at this developmental stage. In addition, treating wild-type neurons with the Slack inhibitor PRX-2904 starting 30 minutes before stimulation also prevented the increase of GluR1 intensity after stimulation. These results support a model where Slack activity plays a direct role in the short-term maintenance of LTP. Slack channels have been shown to regulate local protein synthesis by binding regulators of mRNA translation, and we are currently testing the hypothesis that loss of this activity-dependent translation leads to the observed loss of long-term synaptic potentiation.

Disclosures: S. Gilliat: None. **J. Wu:** None. **L.K. Kaczmarek:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.15/C36

Topic: B.05. Synaptic Plasticity

Title: Histamine H3 Receptor Inverse Agonist Affects Frequency-Specific Network Responses Following Moderate Prenatal Alcohol Exposure

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Abstract: Prenatal alcohol exposure (PAE) disrupts hippocampal circuit development and impairs synaptic plasticity, contributing to long-lasting cognitive deficits. Frequency domain analysis offers a sensitive approach for detecting frequency-specific disruptions and treatment-induced normalization of oscillatory dynamics beyond traditional amplitude-based metrics. Here, we investigated the effects of the histamine H3 receptor inverse agonist SAR-152954, which rescues PAE-related LTP deficits, on spectral power of continuously recorded local field potentials and paired-pulse plasticity in adult male rats exposed to moderate PAE. In vivo electrophysiology was performed in the dentate gyrus at baseline (30-min) and following high-frequency stimulation (HFS) in the perforant path (60-min). Power spectral density (PSD) was computed from 1-s continuous recordings sampled at 1000 Hz and averaging the resulting spectra to estimate frequency-specific power within delta, theta, alpha, beta, and gamma bands for 30-sec epochs. Relative power within each band was calculated as the proportion of total

power and expressed as proportional change from baseline for post-HFS recordings. Paired-pulse responses were recorded at baseline and post-HFS. SAR-152954 or vehicle was administered intraperitoneally (1 mg/kg) prior to recording. At baseline, SAR-152954 significantly increased theta-band power in both SAC and PAE rats, consistent with preparation of hippocampal circuits for efficient encoding of experience. Following HFS, PAE rats exhibited reduced delta-band and increased alpha-band power compared to controls; Comparable, but non-significant, increases in theta, beta, and gamma power were also observed in PAE rats given SAR-152594. These changes emerged only in fractional power metrics, underscoring the sensitivity of frequency-domain analysis to activity-dependent plasticity mechanisms altered by PAE. There were no effects of PAE on paired-pulse ratios. However, SAR-152954 significantly increased paired-pulse ratios at both baseline and post-HFS, suggesting potential reduction neurotransmitter release probability for the first pulse or increased release for the second pulse. This unexpected effect highlights the need for direct measurement of glutamate release to clarify the presynaptic mechanisms underlying the effects of PAE and SAR-152954 on activity-dependent plasticity. These results indicate that SAR-152954 modulates hippocampal oscillatory dynamics in a frequency-specific and experience-dependent manner, particularly in PAE-exposed animals.

Disclosures: M. Goncalves-Garcia: None. **S. Finger:** None. **S. Davies:** None. **D.D. Savage II:** None. **D.A. Hamilton:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.16/C37

Topic: B.05. Synaptic Plasticity

Support: NIH grant NS112706

Title: Hippocampal LTP depend on long term changes in mitochondrial ATP synthase c-subunit leak channel

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Abstract: Long-term potentiation (LTP) of synaptic transmission and structural synapse remodeling underlie hippocampal learning and memory. Here we show that rapid onset long-lasting inhibition of a leak channel in the mitochondrial inner membrane ATP synthase c-subunit (ACLC) is required to initiate and sustain LTP. ACLC inhibition causes a long-term increase in mitochondrial ATP production efficiency demonstrated by an increase in cellular ATP level accompanied simultaneously by a decrease in oxygen consumption. LTP-stimulus induced inactivation of ACLC occurs initially through the rapid recruitment of the anti-cell death protein BCL-xL to the ATP synthase F1 complex and subsequently by the synthesis of the F1

subcomplex that forms an inactivation gate of the ACLC. The resultant localized increase in ATP production close to mitochondria near or within the postsynaptic spine is required for LTP-stimulus induced phosphorylation events and AMPA receptor trafficking. The sequence of synaptic activity followed by leak channel closure evokes LTP-stimulus induced local protein synthesis. In contrast to leak channel inhibition, increased ACLC amount or activity inhibits the change in bioenergetic efficiency, prevents phosphorylation events and hippocampal slice LTP and impairs memory formation *in vivo*. We conclude that long term inhibition of mitochondrial ATP synthase leak is critical and proximal for LTP and memory formation.

Disclosures: P. Licznerski: None. **K.N. Alavian:** None. **E.A. Jonas:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.17/Web Only

Topic: B.05. Synaptic Plasticity

Support: CAPES/PROEX
FAPESP

Title: Early auditory deprivation impacts memory and neurogenesis but not long term potentiation in the hippocampus

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Abstract: The complete maturation of auditory circuits occurs only after the opening of the auditory canal and hearing begins. Evidence suggests that cognitive functions rely on auditory sensory stimuli for full development, but the mechanisms linking hearing loss to higher brain functions remain unclear. This study evaluates the effects of early auditory deprivation on memory and the induction of long term potentiation (LTP). At postnatal day 14 (P14), coinciding with the opening of the auditory meatus, male Swiss mice had their auditory canal obstructed with silicone earplugs. After 30 days of deprivation (P44) auditory thresholds were measured using Auditory Brainstem Response (ABR). Subsequently, the animals were euthanized for brain tissue dissection used to prepare 400µm hippocampal slices. Extracellular recordings were performed in CA1 pyramidal and dentate gyrus (DG) granule neurons. LTP was induced using the theta-burst stimulation (TBS) protocol. Another group of animals underwent the Novel Object Recognition (NOR) and Object Location tests (OLT) and was then euthanized for brain tissue collection. Brain slices of 100 µm and 35 µm thickness were prepared for dendritic spine (Fd Rapid GolgiStain™) and neurogenesis (anti-DCX, Rabbit polyclonal 1:1500) analysis in

DG. Procedures were approved by the Animal Ethics Committee of FMRP (protocol 1290/2024R1). 30 days of deprivation increased auditory thresholds in the Plug group compared to the Sham group (Unpaired t test $p<0.0001$). The Plug group also showed reduced discriminative ability in NOR ($p=0.0005$) and OLT ($p=0.0362$). The TBS protocol effectively induced LTP in CA1 (control $p=0.0022$, sham $p=0.0187$, plug $p=0.0009$) and DG (sham $p=0.0130$, plug $p=0.0160$) in all groups. No significant differences were found between groups in the fEPSP slope before and after LTP induction in the CA1 (One Way ANOVA before $F=3.954(2,26)$/ after $F=1.298(2,23)$) and DG (before $p=0.4530$ / after $p=0.2880$). The afferent volley amplitude did not increase after LTP induction in the CA1 (control $p=0.1404$, sham $p=0.1501$, plug $p=0.2379$) and DG (sham $p=0.8061$, plug $p=0.9732$) in any group. However, the afferent volley amplitude, both before ($p=0.0608$) and after ($p=0.0547$) LTP induction in the DG of the Sham group tended to be higher compared to the Plug group. No differences were found in the number of dendritic spines ($p=0.4797$), but a reduction in neurogenesis ($p=0.0423$) was observed. Our results indicate that early auditory sensory deprivation leads to memory impairments and reduced neurogenesis, but such impairment is not accounted for by long term plasticity capacity in the hippocampus. This work was supported by CAPES/PROEX and FAPESP.

Disclosures: M.M. Migliaccio: None. **L.C. Vanderlei:** None. **N. Benites:** None. **D.M. Filho:** None. **R.M.X. Leao:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.18/C38

Topic: B.05. Synaptic Plasticity

Support: UCC Grant INV3093

Title: Medium-chain polyphosphates exert a modulatory activity on both stimuli-evoked oscillatory activity and synaptic plasticity in the vibrissal primary motor cortex

Authors: I. GUATIBONZA AREVALO[1], A. RAMIREZ ENRIQUEZ[2], P. ARENAS-LÓPEZ[2], A. MORENO PARRA[3], F. A. RUIZ[4], M. MONTILLA RODRÍGUEZ[5], *A. MÚNERA[6]; [1]Dept. of Physiological Sci., [2]Dept. of Biol., Univ. Nacional de Colombia, Bogota, Colombia; [3]Dept. of Biol., Univ. Nacional de Colombia, Bogotá, Colombia; [4]Univ. de Cádiz, Cadiz, Spain; [5]Univ. Cooperativa de Colombia, Villavicencio, Colombia; [6]Physiological Sci., Univ. Nacional De Colombia, Bogotá, Colombia

Abstract: Inorganic polyphosphates (polyP), a group of evolutionarily conserved orthophosphate polymers, regulate diverse cellular processes in both prokaryotic and eukaryotic (either unicellular or multicellular) organisms. Although their role in neural tissue is less known, it has been described that those polymers regulate neuronal metabolism and serve as

neuroprotective agents, and that they act as putative neurotransmitter or gliotransmitter function activating purinergic receptors. We therefore evaluated the effect of intracortical administration of medium-chain polyP (~100 phosphate units) in two concentrations (either 50 or 200 mM) on vibrissal primary motor cortex (vM1) oscillatory activity and synaptic plasticity induced by theta-burst stimulation (TBS) of homotopic commissural projections in anesthetized male Wistar rats. Although intracortical polyP administration did not modify basal oscillatory activity in vM1, it significantly changed the frequency content of whiskerpad and commissural stimulation-evoked potential. While 50 mM polyP increased both commissural- and whiskerpad stimulation-evoked potential, 200 mM polyP increased only commissural-evoked potential in vM1. In addition, polyP administration had a dual effect on vM1 synaptic plasticity. In control subjects TBS applied to commissural projections induced long-term potentiation of both commissural- and whiskerpad stimulation-evoked potentials. In fact, 50 mM polyP enhanced TBS-induced potentiation of whiskerpad- but not commissural stimulation-evoked; by contrast, 200 mM polyP administration enhanced potentiation in commissural-evoked potential, but induced depression of whiskerpad-evoked potential. Our findings show that intracortical medium-chain polyP administration has modulatory effects in the way vM1 processes somatosensory information, and in its synaptic plasticity processes. Such effects may depend on varying degrees of purinergic receptors activation by polyphosphates.

Disclosures: I. Guatibonza Arevalo: None. **A. Ramirez Enriquez:** None. **P. Arenas-López:** None. **A. Moreno Parra:** None. **F.A. Ruiz:** None. **M. Montilla Rodríguez:** None. **A. Múnera:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR376.19/C39

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01HL142129
NIH Grant HL142129-04S1
NIH Grant DK R01105588

Title: Memory impairment and elevated cholesterol: Inflammatory transcription factor STAT4 as a mediator of western diet-associated impairments in synaptic plasticity

Authors: *C. HOLLANDER[1], X.-L. ZHANG[2], M. D'SILVA[3], J. NADLER[3], P. K. STANTON[2];
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Abstract: The relationship between cholesterol and cognitive decline remains an area of ongoing debate. Cholesterol dysregulation is a known risk factor for memory-related disorders such as

Alzheimer's disease (AD), type 2 diabetes mellitus (T2DM), and vascular dementia (VaD). While the cellular mechanisms underlying these remain unclear, high cholesterol can trigger vascular and inflammatory changes that disrupt the blood-brain barrier, damage neurons, and promote plaque formation, all which may drive the pathogenesis of these disorders. During an inflammatory response, cytokine interleukin-12 (IL-12) activates STAT4, an inflammatory transcription factor that promotes pro-inflammatory gene expression and T-helper 1 cell differentiation, potentially contributing to neuroinflammation and impairments in synaptic plasticity and memory storage. To evaluate the impact of elevated cholesterol and STAT4 activation on long-term memory storage, we assessed two forms of activity-dependent long-term synaptic plasticity: long-term potentiation (LTP), the sustained strengthening of synapses following high-frequency stimulation, and long-term depression (LTD), the weakening of synapses in response to low-frequency stimulation. We measured LTP and LTD at CA3-CA1 synapses following Schaffer collateral axon stimulation in hippocampal slices from 24-week-old low-density lipoprotein receptor knockout (LDLr-/-) mice fed either standard chow or a high-fat, high-cholesterol diet (HFD-C) for 16 weeks, which significantly increased serum cholesterol. We then examined whether reducing activated STAT4 levels could protect against any observed HFD-C synaptic plasticity deficits by comparing these mice to genetically modified mice with reduced STAT4 expression in myeloid cells and neurons. Finally, we tested behavioral outcomes across experimental groups using open field, novel object recognition, and Barnes maze tests to evaluate whether these synaptic plasticity deficits correspond with cognitive performance impairment. Our results indicate that elevated serum cholesterol from HFD-C impairs LTP similarly in both sexes of LDLr-/- mice, while LTD shows sex-specific effects, with mild deficits in males and improvement in females. Notably, reducing STAT4 activation preserves synaptic plasticity, protecting both sexes from HFD-C-induced impairments in LTP, and enhancing LTD for both sexes when fed an HFD-C. These findings identify STAT4 as a key mediator of HFD-C induced synaptic plasticity deficits and suggest that its suppression may mitigate cholesterol-related cognitive decline in conditions such as AD, T2DM, and VaD.

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Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR376.20/C40

Topic: B.05. Synaptic Plasticity

Support: MJFF-022867
NINDS R21 NS130841
NINDS R01NS135728

Title: Early and persistent rescue of corticostriatal LTP by in vivo MLi-2 administration in mice carrying a Parkinson's linked LRRK2^{G2019S} mutation

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Abstract: Cognitive impairment is common in Parkinson's (PD) and includes executive functions normally requiring corticostriatal connectivity. In mice, cognitive tasks impaired in PD drive Hebbian forms of corticostriatal synaptic plasticity, such as long-term potentiation (LTP). This raises the idea that PD-associated cognitive impairment may reflect deficits in corticostriatal LTP. Consistent with this, we have shown previously that *Lrrk2*^{G2019S} knockin mice exhibit mild cognitive dysfunction and lack corticostriatal LTP at SPN synapses in dorsomedial striatum (DMS). Moreover, D_1R-SPNs exhibit excessive synaptic accumulation of GluA1 at baseline and lack appropriate cell-surface trafficking of GluA1 that normally follows an LTP stimulus. Here, using both culture and *in vivo* models, we asked if such plasticity deficits and trafficking abnormalities could be rescued by inhibiting the excessive kinase activity of the LRRK2 mutation. We first confirmed in both corticostriatal cocultures and acute striatal slices from mice aged P21 and P60 that LTP of SPN synapses was absent. Acute bath-applied LRRK2 kinase inhibitors to striatal slices or cocultures failed to rescue LTP, despite lowered pRab levels as expected. Time-course analysis of MLi-2 exposure in the cocultures showed that only 14d MLi-2 treatment was sufficient to both reduce surface GluA1 at baseline (to wildtype levels) and rescue LTP as measured by an increase in synaptic GluA1 in response to the LTP stimulus. Using this timecourse as a guide, we fed nursing *Lrrk2*^{G2019S} dams MLi-2-chow for 14d when their pups were P7-21, and we confirmed that MLi-2 delivery significantly decreased pRab levels. Acute striatal slices taken from P21, MLi-2-chow-treated pups showed that corticostriatal LTP was restored to normal (WT-like) levels. To assess the persistence of such early treatment, a second batch of *Lrrk2*^{G2019S} pups was treated with MLi-2 *in vivo* (P7-P21), then returned to regular chow at P21 until they reached P60. Acute striatal slices from this group taken at P60 showed that LTP remained rescued by the early postnatal inhibitor treatment. Ongoing experiments are assessing if inhibitor-based rescue of LTP is associated with normalized surface GluA1 levels and activity-dependent trafficking, and if the timing of *in vivo*-based inhibitor exposure that rescues LTP conforms to a critical period (versus multiple sensitive periods) of intervention. Together, these experiments suggest that mutation-driven alterations in molecular trafficking necessary for dynamic synaptic signaling are the culmination of durable changes that take weeks of inhibitor exposure to normalize.

Disclosures: S. Gupta: None. **A. Tielemans:** None. **G.W. Huntley:** None. **D.L. Benson:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.21/C41

Topic: B.05. Synaptic Plasticity

Support: NSTC 113-2811-B-A49A-031
NSTC 112-2320-B-A49 -008
MOE 113W032101

Title: Investigation of Synaptic Pathology in a Rat Model of Emotional Dysfunction Induced by Varying Intensities of Traumatic Stress

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Abstract: Traumatic stress is a major contributor to emotional dysfunction, including depression and anxiety, which impose significant societal burdens through impaired functioning and increased healthcare use. Current antidepressants remain limited by delayed onset and suboptimal efficacy. Neurobiological evidence links emotional disturbances to disrupted synaptic plasticity and morphology in the prefrontal cortex (PFC), yet the relationship between traumatic stress-induced emotional dysfunction and changes in the synaptic-related mechanisms remain poorly understood. This study explores how different intensities of traumatic stress affect emotional behavior and synaptic pathology in a rat model. Traumatic stress was induced using a footshock paradigm, where rats were subjected to 3, 6, or 10 tone (conditioned stimulus; CS)-shock (unconditioned stimulus; US) pairings to mimic mild, moderate, and severe traumatic experiences, respectively. This approach enables us to examine how varying intensities of traumatic stress affect emotional behaviors and to explore the underlying synaptic mechanisms, particularly within the PFC. The results showed that only the group exposed to severe trauma exhibited depressive-like behaviors, while anxiety-like behaviors were observed across all trauma intensities. Electrophysiological recordings indicated that basal synaptic responses were not affected by different intensities of stress. However, long-term potentiation (LTP), induced by both high-frequency stimulation and theta-burst stimulation, was significantly impaired only in the severe trauma group, suggesting intensity-dependent deficits in synaptic plasticity. Morphological analyses revealed a progressive reduction in apical dendritic branching of prefrontal cortical neurons with increasing trauma intensity, whereas basal dendritic structure remained unchanged. Furthermore, a significant decrease in spine density was observed exclusively in the severe trauma group. Notably, the proportion of spine types was also altered following severe trauma, indicating structural remodeling associated with synaptic dysfunction. Severe traumatic stress induces depressive-like behaviors and causes synaptic plasticity and structural abnormalities in the prefrontal cortex, while varying stress intensities generally evoke anxiety-like behaviors without affecting basal synaptic function. This suggests that trauma intensity critically determines the severity of emotional and synaptic pathology.

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Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.22/C42

Topic: B.05. Synaptic Plasticity

Support: KGM4562532

Title: Vps26b improves motor learning ability in mptp-induced parkinson's disease model via glutamate receptor recycling-dependent synaptic plasticity in primary motor cortex

Authors: ***T. T. NGUYEN**[1], J. SEONG[2], J. SEO[3], J. WON[4], Y. LEE[5];
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Abstract: The Parkinson's disease (PD) patients have motor learning impairment related to reducing synaptic plasticity in primary motor cortex (M1). Cortical synaptic plasticity is associated with glutamate receptor trafficking and phosphorylation of glutamate receptor regulated by dopamine via PKA signaling. However, studies using PD animal models have shown that dopaminergic degeneration from VTA projecting to M1 might cause insufficient phosphorylated AMPA-type glutamate receptor GluA1. As a result, GluA1 cannot be inserted to cell membrane, leading to impairment of synaptic plasticity and motor learning. Several studies have tried to restore motor learning ability using other modulators such as acetylcholine, serotonin and GABA to compensate for the lack of dopamine, but only partial restoration has been achieved.Here, in the strategy of increasing motor learning ability for MPTP-induced PD mice model, we focus on supporting GluA1 trafficking by overexpression of VPS26B, a component of retromer which is a molecular trafficking machine.We found in the MPTP-induced PD mice model that pre-overexpression with VPS26B into M1 increases surface and total glutamate receptor in M1. This result may explain why levels of presynaptic marker Synaptophysin, and postsynaptic marker PSD-95 were protected in VPS26B overexpressing mice groups even under MPTP injection condition. Moreover, VPS26B knock out mice had the shortest time on rotarod test proves that reducing VPS26B may contribute to the pathology of clinical PD. Noteworthy, VPS26B alleviated TH+ neuron loss in VTA, substantia nigra (SN) and TH+ fiber loss in the M1 as well as striatum. We anticipate our research to be a starting point for using retromer in general and VPS26B in particular as a valuable treatment for PD.Key words : *Parkinson's disease, retromer, VPS26B*

Disclosures: T.T. Nguyen: None. **J. Seong:** None. **J. Seo:** None. **J. Won:** None. **Y. Lee:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.23/C43

Topic: B.05. Synaptic Plasticity

Title: Brief transcranial focused ultrasound stimulation causes lasting modifications to the synaptic circuitry of the rat hippocampus

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Abstract: Brief transcranial focused ultrasound (tFUS) is used in cognitive mapping, where it is assumed that the intervention itself does not cause lasting modifications to the underlying networks being targeted. However, how post-stimulation ('offline') effects impact the dynamic function of neural circuits is largely unknown. We found that 40 s of low-intensity tFUS targeting the hippocampus in 12-week-old male and female rats *in vivo* led to lasting changes in synaptic circuitry. Following sham or tFUS stimulation, acute hippocampal slices were immediately collected from treated animals (*n= 6 (sham) vs 6 (tFUS)*) and *ex vivo* field electrophysiology assays were performed at CA3-CA1 synapses. When compared with sham stimulated tissue, CA3-CA1 synapses in ultrasound stimulated hippocampi showed elevated basal transmission and increased susceptibility to long-term potentiation (LTP); both effects lasted for the duration of experiments (1 - 8 hours post-stimulation). These findings mimicked *in vitro* experiments in which identical changes to CA3-CA1 synaptic circuitry were observed in acute hippocampal slices following slice exposure to 40 s of ultrasound or sham stimulation. Notably, the application of an AKT inhibitor to the *in vitro* hippocampal slices before ultrasound stimulation abolished offline changes to LTP, suggesting that a canonical AKT-dependent metaplastic process underlies this ultrasound-mediated effect to hippocampal circuitry. Together, these results indicate that brief ultrasound stimulation can induce persistent changes to synaptic function, driving metaplastic processes beyond the duration of the stimulus itself. Such effects could be leveraged in the use of tFUS in therapeutic neuromodulation and highlight the capacity for brief ultrasound stimulation to induce significant offline effects.

Disclosures: W.W. Watts: None. **R. Cuthell:** None. **D.J. Whitcomb:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.24/C44

Topic: B.05. Synaptic Plasticity

Title: Short-interval Intracortical Inhibition and Facilitation in Corticospinal Excitability to the Lower Limb Following Acute Intermittent Hypoxia

Authors: *L. R. DEMINSKI[1], J. L. NESBIT[1], A. K. POLLET[2], A. Q. TAN[3];
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Abstract: Short-interval Intracortical Inhibition and Facilitation in Corticospinal Excitability to the Lower Limb Following Acute Intermittent Hypoxia AUTHOR BLOCK: L.R. DEMINSKI[1],

J.L. NESBIT[1] A.K. POLLET[1], *A.Q. TAN[1,2] [1]Integrative Physiology., [2]Center for Neuroscience, Univ. of Colorado, Boulder, CO Motor recovery following neurological injury such as spinal cord injury (SCI) relies on adaptive neuroplasticity within spared descending pathways. Previous studies show that breathing mild bouts of low oxygen air, known as acute intermittent hypoxia (AIH), with task-specific training improves motor recovery. Emerging evidence from humans indicates that AIH-induced increases in corticospinal excitability (CSE) may underlie these motor improvements. However, the mechanisms by which AIH facilitates these changes in CSE are unclear. Preclinical studies from spinally injured rats show that AIH promotes brain-derived neurotrophic factor (BDNF) expression, which is associated with increased motoneuron excitability and motor recovery. To further explore the mechanisms of AIH, we examined the modulation of short interval intracortical inhibition (SICI) and facilitation (SICF) represented by changes in motor evoked potentials using validated double pulse transcranial magnetic stimulation (TMS) protocols. We hypothesize that SICI and SICF responses will be altered by AIH, producing reduced inhibition and increased facilitation. During AIH treatment, participants breathed 90-second bouts of hypoxic air (9% O_2) alternated with 60 seconds of normoxic air (21% O_2) for 15 episodes. To determine the active motor threshold, (AMT) we used the lowest stimulus that elicited ≥ 750 μV MEPs in four out of seven trials during an activation to 20% of the participant's maximal voluntary contraction. The SICI protocol involved a subthreshold stimulus (60% AMT) followed by a test stimulus (100% AMT), while the SICF protocol utilized the test stimulus (100% AMT) followed by a subthreshold stimulus (90% AMT). Preliminary results show a suppressed SICI and enhanced SICF in the lower limb following a single AIH exposure. These findings contrast with prior evidence showing no change in SICI and SICF in the upper limb using resting motor threshold-based protocols. These observations suggest that AIH may exert limb-specific effects on intracortical excitability that reflect differences in how motor pathways are organized in the upper versus lower limb. Examining the mechanisms behind AIH induced plasticity in humans can help identify who is likely to respond and support the clinical translation of AIH as adjuvant to enhance motor recovery for people with neurological injury.

Disclosures: L.R. Deminski: None. **J.L. Nesbit:** None. **A.K. Pollet:** None. **A.Q. Tan:** A. Employment/Salary (full or part-time): University of Colorado Boulder.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.25/C45

Topic: B.05. Synaptic Plasticity

Support: Grants-in-Aid for Scientific Research B (No. 16H05129) from the Ministry of Education, Culture, Sports, Science and Technology of Japan
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Research Grant from Kao Health Science Research Foundation (2024)
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Title: Super bursts reorganize hippocampal firing and promote synaptic diversity during stress-induced memory encoding

Authors: *Y. YANG[1], J. ISHIKAWA[1], D. MITSUSHIMA[1,2];
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Abstract: The formation of episodic memory depends on activity-dependent synaptic plasticity in the hippocampal CA1 region. While artificial high-frequency stimulation is known to induce plasticity (Bliss & Lømo, 1973), the endogenous neuronal firing patterns that trigger such changes during memory encoding remain poorly understood. We identified a novel firing pattern, termed the "super burst", which spontaneously emerges in the CA1 region of freely moving rats during emotionally salient experiences. Super bursts are defined as transient high-frequency events in which multi-neuron firing exceeds three standard deviations above baseline, detected via chronically implanted, depth-adjustable multi-unit electrodes. These events precede an increase in sharp-wave ripple activity, suggesting a role in memory encoding. To examine their functional significance, we developed a real-time detection and closed-loop intervention system by anterior commissure stimulation. We then analyzed miniature excitatory and inhibitory synaptic currents (mEPSCs and mIPSCs) in CA1 pyramidal neurons using whole-cell patch clamp recordings from acute slices. Allowing super bursts to occur led to significant increases in both the amplitude and frequency of excitatory and inhibitory synaptic inputs, indicating enhanced synaptic diversity. In contrast, selective suppression of super bursts attenuated these changes, suggesting a causal role in driving plasticity. Entropy analysis further revealed greater informational diversity in the amplitude and frequency domains in stressed animals compared to those deprived of super bursts. Behaviorally, suppression of super bursts resulted in reduced freezing responses, indicating impaired memory formation. These findings support the hypothesis that super bursts induce bidirectional synaptic strengthening, expanding the dimensionality of synaptic diversity. This mechanism may enhance the precision and stability of memory traces under emotionally charged conditions. Ongoing studies are investigating the behavioral consequences and cell-type specificity of this phenomenon.

Disclosures: Y. Yang: None. **J. Ishikawa:** None. **D. Mitsushima:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.26/C46

Topic: B.05. Synaptic Plasticity

Support: NIH Grant DA049260
BYU Mentoring Environment Grant

Title: Lsd restores morphine-eliminated synaptic plasticity in the ventral tegmental area and reverses morphine-induced behavioral preference

Authors: *J. G. EDWARDS[1], M. VON GUNTEN[2], T. RUSSELL[1], **C. BERRETT**[1]; [2]Neurosci., [1]Brigham Young Univ., Provo, UT

Abstract: Psychedelics are emerging as a promising treatment option for a range of neuropsychiatric disorders, including substance use disorders. One potential mechanism underlying their therapeutic benefits may involve a reversal of maladaptive plasticity induced by drug exposure. Here, we identify physiological, behavioral, and epigenetic impacts of lysergic acid diethylamide (LSD) on morphine-treated mice. Male and female CD1 GAD67-GFP knock-in mice (P21-P65) received intraperitoneal (IP) morphine injections every other day for seven days. Some mice received low-dose LSD (0.03 mg/kg) along with morphine injections, while others got a high dose of LSD (0.3 mg/kg) on the day after morphine injections concluded. A single treatment of LSD ($n=10$, $p < 0.05$; ANOVA), or 4 microdoses of LSD ($n=9$, $p < 0.05$; ANOVA), cause accelerated extinction of morphine-induced conditioned place preference. *Ex vivo* whole-cell electrophysiology revealed that excitatory long-term depression (LTD) synaptic plasticity, which was eliminated in VTA GABA neurons following morphine exposure, was restored 24 hours after a single high dose of LSD ($n=7$ cells from $n=4$ mice, $p < 0.05$; ANOVA). Unexpectedly, low-dose LSD treatments did not fully restore LTD ($n=8$ cells from $n=5$ mice; $p> 0.05$; ANOVA), so the accelerated extinction of morphine-induced CPP caused by low-dose injections may have resulted from mechanisms unrelated to plasticity. To explore the impact of LSD on potential epigenetic changes, whole-brain DNA methylation analysis in morphine-treated mice that received either saline or LSD post-morphine treatment revealed significant differences in methylation profiles associated with LSD treatment. Our work not only identifies LSD as a possible therapeutic for SUD but also proposes an underlying mechanism by which LSD combats it. Collectively, these findings suggest that LSD may reverse or prevent morphine-induced changes in reward circuit plasticity and attenuate measures of morphine preference.

Disclosures: J.G. Edwards: None. **M. Von Gunten:** None. **T. Russell:** None. **C. Berrett:** None.

Poster

PSTR376: Long-Term Synaptic Plasticity: Induction and Modulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.27/C47

Topic: B.05. Synaptic Plasticity

Support: NIH Grant DA049260
NIH Grant DA038092
BYU Mentoring Environment Grant

Title: Chronic morphine differentially impacts glutamatergic synaptic plasticity in the ventral tegmental area of adults versus adolescent mice

Authors: *C. BERRETT[1], R. BONNEY[2], I. OSTLUND[3], M. VON GUNTEN[4], I. CRESS[1], J. G. EDWARDS[5];
[1]Brigham Young Univ., Provo, UT; [2]Brigham Young Univ., Provo, UT, ; [3]Physiol. and Developmental Biol., [4]Neurosci., [5]Cell Biol. and Physiol., Brigham Young Univ., Provo, UT

Abstract: Opioid overdose causes 7 of 10 drug-related US deaths per year, yet the precise mechanisms underlying opioid addiction are unknown. Opioid addiction is linked to maladaptive plasticity in the ventral tegmental area (VTA) due to mu opioid receptor (MOR) agonism. We identified excitatory synapses onto VTA GABA cells contain MORs in addition to cannabinoid 1 (CB1) receptors. Our previous work identified a novel form of CB1-dependent long-term depression (LTD) at excitatory synapses onto VTA GABA cells. To further characterize this synapse, we explored the role of MOR function using ex vivo whole-cell physiology in young (P15-40) and adult (P70-150) CD1 GAD67-GFP mice, allowing us to identify VTA GABA cells. MOR activation with selective agonist DAMGO (0.03 μM) depresses EPSC amplitude in young (33.0 ± 9.79%; n=7, $p < 0.0001$; ANOVA) and adult (32.4±8.9%; n=5, $p < 0.0001$; ANOVA) mice, demonstrating their expression at this synapse, but also unexpectedly DAMGO occludes LTD (n=5, $p > 0.5$; ANOVA). However, the MOR-selective antagonist, naloxone, did not prevent LTD induction in young mice (n=6, $p < 0.0001$; ANOVA), unlike CB1 blockade. While both CB1 and MORs induce synaptic depression, only CB1 receptors are necessary for LTD. Similarly to chronic THC, chronic morphine administration eliminates LTD in young mice ($p>0.05$ compared to baseline; n=7); however, following 7 days of withdrawal, LTD reappears (36.4 ± 4.9% compared to baseline, n=5, $p<0.001$; ANOVA). This illustrates that morphine-induced occlusion of glutamatergic LTD to VTA GABA cells is reversible, like VTA dopamine neurons in young mice. Opiate-induced impact on plasticity also exhibited important age-related characteristics. Unlike young mice where chronic morphine eliminated LTD in all cells, LTD in adults was preserved in ~50% of cells (34.3 ± 7.9% compared to baseline, n=5, $p<0.001$; ANOVA) and eliminated in the other 50% ($p>0.05$ compared to baseline, n=7). In addition, after a 7-day morphine withdrawal period LTD was re-established in only ~50% of adults (LTD, 38.3±7.37%, n=3, $p<0.001$; ANOVA; no LTD, $p>0.05$, n=5), while it was restored in 100% of young mice. This suggests age should be considered in synaptic plasticity studies, particularly those carried out in drug exposure paradigms. Furthermore, the shared expression profile and plasticity impact of endocannabinoids and opioids at the synapse indicate an overlap in the two pathways and a novel mechanism by which opioids may contribute to drug dependence in the VTA. Understanding plasticity in various synapses, including the VTA, and at various ages, can provide a background for possible therapeutic interventions for treating opioid addiction.

Disclosures: C. Berrett: None. **R. Bonney:** None. **I. Ostlund:** None. **M. Von Gunten:** None. **I. Cress:** None. **J.G. Edwards:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.01/C48

Topic: B.08. Epilepsy

Support: NIH R01 NS066974

Title: Serotonergic Modulation of Cortical Arousal During Focal and Generalized Seizures with Impaired Consciousness

Authors: *J. LIU[1], V. DOAN[1], J. A. CARDIN[2], H. BLUMENFELD[1];
[1]Dept. of Neurol., Yale Univ. Sch. of Med., New Haven, CT; [2]Dept. of Neurosci., Yale Univ., New Haven, CT

Abstract: Patients with focal and generalized seizures often experience loss of consciousness, which frequently extends into the post-ictal period. In focal limbic seizures, impaired arousal is associated with suppression of subcortical arousal systems, including brainstem serotonergic pathways. Disruption in these circuits contributes not only to post-ictal impaired consciousness but also to altered cardiorespiratory function and heightened risk of morbidity and mortality. Awake rodent studies from our lab show that in mice, focal seizures induce slow-wave activity in the orbitofrontal cortex (OFC) with reduced cholinergic arousal due to direct inhibition and indirect deexcitation of the nucleus basalis; multi-unit activity in the locus coeruleus (LC) also decreases. In rats, focal and generalized seizures suppress single-unit activity in the medullary raphe. We hypothesize that serotonin (5-HT) levels in the OFC during focal and generalized seizures decrease due to reduced serotonergic output from the medullary raphe and contribute to cortical arousal suppression. We apply an awake mouse model in which focal and generalized seizures are induced by electrical stimulation of the hippocampus. Local field potentials (LFPs) are recorded from the hippocampus and orbitofrontal cortex (OFC), with behavioral monitoring of running and licking. To assess neuromodulatory changes, we use fiber photometry to measure 5-HT level in the OFC under two experimental conditions: (1) focal temporal lobe seizures and (2) generalized seizures. During focal-induced seizures, the OFC shows slow wave activity and arrested running and licking behavior that quickly returns to normal licking and running (< 30s after seizure offset). 5-HT levels showed decreases in OFC during focal seizures. During generalized seizures, there was poly-spike and wave epileptiform activity in the OFC, and the 5-HT level (5 seizures) showed a larger and more sustained decrease during and after seizures than in focal seizures. Behaviorally, in generalized seizures, the animal showed extended impaired running and licking (>30s after seizure offset). Our results show during focal and generalized seizures, there is a decreased 5-HT innervation of cortical arousal in ictal and post-ictal periods. Further work includes GCaMP measure of serotonergic neuronal activity in brain stem using fiber photometry, and sensing of other neurotransmitters in the OFC in the same experimental scenarios. We aim to uncover the mechanisms responsible for impaired consciousness during ictal and postictal periods in focal and generalized seizures and potentially leading to the development of innovative treatments.

Disclosures: J. Liu: None. **V. Doan:** None. **J.A. Cardin:** None. **H. Blumenfeld:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.02/C49

Topic: B.08. Epilepsy

Support: NIH/NINDS R37NS100901

Title: To discharge or not to discharge: spike-wave discharge probability in three absence epilepsy mouse models modulated by behavioral tasks

Authors: *S. CHOPRA[1], S. SUMSKY[1], Y. ZHENG[1], J. LIU[1], H. BLUMENFELD[2];
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Abstract: Spike and wave discharges (SWD) are commonly associated with absence seizures, a generalized seizure disorder with lapses in consciousness predominantly seen in children. The mechanisms that underlie impaired consciousness remain uncertain, largely due to inadequate animal models for more invasive exploration. Behavioral tasks can be used as a proxy indicator of level of consciousness as they require at least some arousal and engagement. Previous work done in rats validated task-associated behavioral impairments seen in patient cohorts. Our goal was to develop a lick-on-cue behavior task for mice to establish a paradigm that can be used to interrogate perturbed cortical signaling pathways associated with lapses in behavioral responsiveness in rodents. 8 - 15-week-old male and female mice from three genetic models of absence epilepsy (C3H/HeJ, HEJ/FEJ, and SCN8A) were implanted with tripolar intracranial EEG electrodes in the frontal and parietal cortex with a ground electrode mid-cerebellum. Following recovery, the mice were head-fixed on a running wheel and trained on a lick-on-click auditory task (278 total recording sessions from 12 mice). We found that all three strains of mice had different SWD frequencies at baseline with 11.55 SWDs/hr in the C3H/HeJ cohort (n=3), and 0.93 SWDs/hr in the HEJ/FEJ cohort (n=3), and 105.72 SWDs/hr in the SCN8A cohort (n=6). We found that the SWD frequency was markedly reduced with prolonged water deprivation associated with task performance in the SCN8A cohort. However, SWDs could be rescued with increased time on the running wheel and increased random time interval between cue administrations. Overall, we found that water deprivation decreased SWD frequency across all three models. With further work utilizing cortical network recording methods, this paradigm can help uncover the changes in signaling pathways that underlie the loss of behavioral responsiveness during SWDs. Ultimately, these investigations may shed light on fundamental mechanisms of impaired consciousness in epilepsy, leading to improved treatments for this disorder.

Disclosures: S. Chopra: None. **S. Sumsky:** None. **Y. Zheng:** None. **J. Liu:** None. **H. Blumenfeld:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

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Program #/Poster #: PSTR377.03/C50

Topic: B.08. Epilepsy

Support: This work was supported by NIH R01 NS066974

Title: Ventral Tegmental Area Dopaminergic Circuitry Dynamics During Focal Limbic Seizures with Impaired Consciousness

Authors: Y. ZHENG, J. LIU, J. LI, S. CHOPRA, T. YADAV, S. SUMSKY, *H. BLUMENFELD;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Focal limbic seizures, despite their localized onset, can disrupt widespread cortical networks, leading to impaired consciousness. Ventral tegmental area (VTA), a region rich in dopaminergic (DA) neurons, promotes consciousness in normal conditions and exhibits decreased connectivity in patients with temporal lobe epilepsy. The role of dopaminergic neurons in the VTA (VTA^{DA}) in impaired consciousness during seizures remains unknown. Here, we investigated the dynamics of VTA dopaminergic circuitry in a novel awake mouse model of focal limbic seizures with reduced cortical electrophysiological and behavioral arousal. Local field potentials were recorded from the hippocampus and the orbitofrontal cortex. To assess behavioral arousal during seizures, we measured licking responses to auditory stimuli and wheel-running speed. We first recorded multiunit activity in the VTA during seizures while simultaneously evaluating arousal. We observed decreased licking responses and running wheel speed and increased slow wave activity in the orbitofrontal cortex during focal limbic seizures. Overall neuronal firing rates were reduced in the VTA (n = 6 seizures from 6 mice). Spike sorting revealed a divergence in activity: putative DA neurons exhibited increased firing, whereas putative non-DA neurons showed decreased firing. Next, we measured calcium dynamics in VTA^{DA} using fiber photometry in DAT-Cre mice, which demonstrated elevated calcium activity in VTA^{DA} during focal limbic seizures with impaired arousal (n = 14 seizures from 5 mice). Lastly, using genetically encoded DA sensors and fiber photometry, we observed a selective increase in dopamine levels in the medial prefrontal cortex during seizures (n = 8 seizures from 3 mice). Our findings reveal apparently paradoxical increases in VTA^{DA} and their medial prefrontal cortical projections during focal limbic seizures. Ongoing work in the orbitofrontal cortex, where we observed slow wave activity consistent with decreased arousal, may reveal the potential role of DA modulation of arousal in this network. Overall, our results underscore the pivotal role of VTA neuromodulation in focal limbic seizures. With further work, this pathway could emerge as a promising therapeutic target to restore consciousness during seizures.

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Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

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Program #/Poster #: PSTR377.04/C51

Topic: B.08. Epilepsy

Support: NIH/NINDS R37NS100901

Title: Alteration of Oscillatory Evoked Potentials in Anterior Insular Cortex by Spike-wave Discharges Underlie Auditory Processing Disruption During Absence Seizures

Authors: *S. SUMSKY[1], R. ASHMEADE[3], J. LIU[4], Y. ZHENG[2], B. F. GRUENBAUM[5], C. P. MCCAFFERTY[6], H. BLUMENFELD[2];
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Abstract: Absence epilepsy features brief episodes of impaired consciousness with generalized spike-wave discharges (SWDs) on EEG. The neural mechanisms, particularly the role of specific brain regions in disruption of sensory processing during SWDs, remain unclear. The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) model, which mimics human absence seizures, is ideal for studying these dynamics. Previous studies showed that sensory behavioral responses are disrupted during SWDs, yet evoked neuronal activity and event-related potentials (ERPs) in the primary somatosensory cortex remain unaffected, suggesting the disruption originates elsewhere. Here, we investigate disruption of auditory sensory-motor processing in absence seizures, hypothesizing a critical role for the anterior insular cortex (AIC), a region involved in sensory-motor integration. We conducted in vivo electrophysiological recordings in awake, freely behaving GAERS during an auditory response task. Bipolar electrodes recorded auditory ERPs from the primary auditory cortex (A1) and AIC in 10 GAERS and 10 Wistar non-epileptic controls. We analyzed 1143 auditory stimuli at baseline, 1112 during SWDs, and 1058 from controls. Operant-conditioned lick responses to auditory stimuli were consistent at baseline and in controls but disrupted in >99% of SWDs. Grand-averaged recordings showed normal short-latency ERPs in A1, preserved during SWDs. In contrast, AIC recordings revealed polyphasic oscillations with altered amplitude and frequency. In Wistar controls, stable, high-amplitude oscillations occurred at 8-9 Hz. In GAERS at baseline, amplitude decreased, and frequency shifted to 10-12 Hz. During SWDs, oscillations were further distorted, dropping to 4-6 Hz. Synchrony and oscillatory activity are thought to mediate communication between brain regions, integrating multimodal sensory signals. Our findings suggest that the AIC's auditory evoked oscillatory signal, critical for sensory integration, is disrupted in GAERS at baseline, and further disrupted during SWDs despite normal A1 ERPs in all conditions. These results highlight

a novel auditory evoked potential in the AIC with distinct oscillatory characteristics, offering a potential mechanism for sensory processing disruption in absence epilepsy.

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Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

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Program #/Poster #: PSTR377.05/C52

Topic: B.08. Epilepsy

Support: NIH Grant R01NS129784

Title: Determining the efficacy of gene replacement therapy in a mouse model of SLC35A2 deficiency

Authors: *N. SHINAGAWA[1], K. WU[1], H. YOON[2], L. WARREN[3], T. A. BEDROSIAN[2]; [1]Inst. of Genomic Medicine, Nationwide Children's Hosp., Columbus, OH; [2]Inst. for Genomic Med., Nationwide Children's Hosp., Columbus, OH; [3]Inst. for Genomic Medicine, Nationwide Children's Hosp., Columbus, OH

Abstract: The *SLC35A2* gene located on the X chromosome encodes a UDP-galactose transporter that localizes to the endoplasmic reticulum and Golgi body. Recently, somatic variants in *SLC35A2* have been implicated as a prominent cause of mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE), a histopathological entity associated with drug-resistant epilepsy. The development of detrimental phenotypes is believed to be caused by defects in glycosylation; however, the exact mechanisms leading to disease remain unknown. Seizures associated with SLC35A2 deficiency tend to respond poorly to anti-seizure medication due to their drug-resistant nature. For these patients, the standard treatment remains surgical resection of the epileptic focus. Although some patients respond well to surgery, surgical resection carries the risk of damaging vital areas of the brain and cannot be performed when the epileptic focus coincides with a region of high functional importance. Therefore, there is a critical need for an effective medical treatment for seizures caused by *SLC35A2* variants. Our lab previously developed a novel mouse model in which *Emx1*-mediated Cre recombinase expression knocks out *Slc35a2* in forebrain neural progenitors. These mice recapitulate the phenotypes seen in patients with mosaic SLC35A2 deficiency. As *Slc35a2* loss-of-function was sufficient to cause seizures and MOGHE-like phenotypes in mice, we hypothesized that gene replacement would be successful in rescuing these phenotypes. We designed recombinant adeno-associated viral vectors (AAVs) containing *Slc35a2* and mCherry expressed under the CBA promoter and packaged in an AAV8 capsid. We injected these AAVs into the lateral ventricles of postnatal day 0 mice. Efficient transduction of neurons and glial cells in the cerebral cortex was confirmed *in situ* through immunofluorescenc. Gene expression of

Slc35a2 measured through RT-qPCR demonstrates a significant rescue in mice treated with AAVs containing *Slc35a2*-mCherry compared to those treated with AAVs encoding mCherry only. Conditional knockout male mice treated with AAVs have significantly improved survival compared to their littermates injected with the control vector. In addition, treated males show a dose-dependent rescue in their weight profiles, with weights in the group injected with the highest dose tracking with those of wildtype mice. Although much more work establishing the safety and efficacy of gene replacement therapies for disease caused by *SLC35A2* variants is currently ongoing, the data demonstrate the potential for *Slc35a2* gene replacement to rescue phenotypes in SLC35A2-deficient mice.

Disclosures: N. Shinagawa: None. **K. Wu:** None. **H. Yoon:** None. **L. Warren:** None. **T.A. Bedrosian:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

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Topic: B.08. Epilepsy

Support: Nationwide Children's Hospital Office of Trainee Affairs Postdoctoral Idea Award

Title: Endoplasmic Reticulum Stress in the Cortex and White Matter of Slc35a2 Conditional Knockout Mice

Authors: *S. A. DOW, N. SHINAGAWA, T. A. BEDROSIAN;
Nationwide Children's Hosp., Columbus, OH

Abstract: One-third of children with epilepsy experience drug-resistant seizures, yet the underlying epileptogenic mechanisms remain unclear. Recently, loss-of-function somatic variants in the nucleotide sugar transporter gene *SLC35A2* have been implicated in epilepsy in both human patients and rodent models. As seen in congenital disorders of glycosylation, loss of SLC35A2 leads to impaired glycosylation, which can induce the accumulation of misfolded proteins, or endoplasmic reticulum (ER) stress. Importantly, increased ER stress and activation of the compensatory unfolded protein response (UPR) can disrupt key neurodevelopmental processes. These findings point to a potential role for ER stress in *SLC35A2*-related epilepsy, but this relationship remains unexplored. Here, we hypothesized that *Slc35a2* loss drives ER stress in the cortex. To test this, we collected cortex from male and female *Slc35a2*-floxed (control) and *Emx1*-Cre-*Slc35a2* cKO (cKO) mice, a mouse model that recapitulates spontaneous seizures, at postnatal day 21 (n=2-9/group). RT-qPCR analysis revealed both male and female cKO mice displayed decreased *Slc35a2* expression (75% reduction cKO vs. control males, 50% cKO vs. control females, $p<0.05$) in the cortex. Interestingly, cKO males exhibited 40% decrease in ER chaperone *Grp78* ($p<0.05$) and an increase in UPR marker *Pdi* ($p=0.06$) versus control males.

When examining cKO females, sexual dimorphism in ER stress was clear. Specifically, UPR indicator *Perk* was upregulated in female cKO cortex (vs. control, $p<0.05$) by 17% while no change was observed in *Grp78* or *Pdi*. We next examined the localization of ER stress marker expression related to *Slc35a2* loss using 10X Genomics Visium Spatial Gene expression analysis in control and cKO male mice (n=1-2/group). We choose to focus on males given that the cKO phenotype is more severe than in females. Spatial transcriptomic analysis paralleled our RT-qPCR findings ($p<0.001$ for each marker), indicating downregulated *Grp78* in both cortex (14%) and white matter (18%) of cKO males compared to control. In line with this, gene expression of several UPR markers was upregulated in cKOs in cortex (*Atf6:* 42%, *p58ipk:* 56%*, Pdi:* 15%) and white matter (*Atf6:* 74%*, p58ipk:* 29%, *Pdi:* 18%). Intriguingly, cKO males also displayed decreases in UPR markers in cortex (*Perk:* 17%, *Ire1α:* 0.6%) and white matter (*Perk:* 22%, *Ire1α:* 5%, $p=0.07$), which may indicate negative feedback regulation of the UPR. Our findings suggest 1) *Slc35a2* loss elicits ER stress in the cortex and white matter, and 2) *Slc35a2*-related ER stress is sexually dimorphic. Collectively, our data may point to ER stress as a potential mediator for *SLC35A2*-related epilepsy.

Disclosures: S.A. Dow: None. **N. Shinagawa:** None. **T.A. Bedrosian:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

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Program #/Poster #: PSTR377.07/C54

Topic: B.08. Epilepsy

Support: SECIHTI CBF2023-2024-1804

Title: Effect of exogenous beta-hydroxybutyrate on acute seizure-induced pyroptosis in the hippocampus of rats

Authors: O. GUZMAN-GARCIA[1], *O. F. MERCADO-GOMEZ[3,1], V. ARRIAGA-AVILA[2], R. GUEVARA-GUZMAN[4];
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Abstract: Neuroinflammation is a crucial factor in the development and progression of neurodegenerative and neurological diseases such as epilepsy. Recent studies have indicated that pyroptotic programmed cell death has an essential role in the early stages of epileptogenesis and contributes significantly to establishing a pro-inflammatory environment. Previous studies have shown that the ketone bodies such as beta-hydroxybutyrate (BHB) can downregulate inflammasome activation, thereby reducing the release of pro-inflammatory cytokines like IL-1 beta. The present work aimed to investigate whether injection with exogenous D-beta-

hydroxybutyrate (BHB) can prevent pyroptotic cell death through the inhibition of inflammasome activation induced by seizures. We employed the pilocarpine-induced status epilepticus (SE) model. A lithium chloride solution (127mg/kg i.p.) was injected 18 hours before pilocarpine injection. Subsequently, a methylscopolamine solution (1mg/kg s.c.) was injected 30 min before pilocarpine. In the BHBSE group, a BHB solution (4mM/kg i.p.) was injected 30 min before SE induction, while the PBSSE group received an injection of the vehicle (phosphate buffered saline, PBS). Ninety minutes later, the seizures were stopped with a midazolam injection (2 mg/kg i.m.). The PBS control and BHB animals only received a saline injection instead of a pilocarpine injection. All experimental animals were euthanized at 24 h, and the hippocampi were dissected and homogenized in RIPA buffer so the proteins could be separated by PAGE and transferred to nitrocellulose membranes for immunoblotting. Preliminary results showed a tendency to decrease the content of proteins related to pyroptosis such as NLRP3, Caspase 1, and Gasdermin D in rats injected with BHB compared to those that only underwent SE. These findings suggest that pretreatment with BHB may downregulate proteins associated with pyroptotic programmed cell death, potentially leading to a reduction in the pro-inflammatory environment that contributes to seizure development.

Disclosures: O. Guzman-Garcia: None. **O.F. Mercado-Gomez:** None. **V. Arriaga-Avila:** None. **R. Guevara-Guzman:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.08/C55

Topic: B.08. Epilepsy

Title: Interluekin 1 type-1 receptor modulates seizure-induced neuronal injury and death associated with repeated generalized tonic/clonic seizures in a transgenic mouse model of adult-onset epilepsy

Authors: T. DILLON, A. SILVERSTEIN, ***C. ISGOR**;
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Abstract: The pro-inflammatory cytokine interleukin-1 beta is elevated in clinical models of epilepsy, implicating several inflammatory pathways in pro-convulsive synaptic plasticity. Dysregulations in hippocampal dentate granule neuron (DGN) gating properties may create a permissive synaptic environment that spreads hyperexcitability in epileptogenic forebrain circuits. The interleukin-1 type 1 receptor (IL-1R1) is abundantly present in DGNs. We study a transgenic mouse that overexpresses the brain derived neurotrophic factor under the CAM kinase II alpha promoter in the forebrain as a model of adult-onset epilepsy (termed TgBDNF mice). TgBDNF mice develop generalized tonic/clonic seizures (GTCSs) at ~3 months of age in response to tail lifting/ cage agitation, which progressively intensify evidenced by prolonged durations of post-ictal generalized cortical suppression (PGES) with successive GTCSs,

indicative of increased risk of death. In advanced stages, mice expire following a very prolonged PGES. In previous work, we have shown that progressive increase in PGES and heightened risk for death are associated with hypertrophy in forebrain structures and a specific loss of neurons in the brainstem arousal circuits. In the present study we test the hypothesis that IL-1R1 is a pro-epileptogenic modulator of brainstem-cortical networks in the TgBDNF mouse model of adult-onset epilepsy. We bred the TgBDNF mice with a strain that had IL-1R1 constitutively knocked out (IL-1R1 KO). About half of the resultant offspring showed suppression of epileptogenesis entirely, and the remaining half progressed to GTCS+ status. The seizure threshold was increased in the seizure prone TgBDNF with IL-1R1 KO, evidenced by requiring increased number of seizure induction sessions to manifest the first GTCS. Furthermore, animals that did progress to repeated GTCS status did not develop prolonged PGES, but instead recovered from generalized seizures rapidly. Morpho-behavioral analyses showed that hypertrophy of anterior cingulate cortex and hippocampal dentate gyrus along with neuron loss in brainstem nucleus pontis oralis were reversed in TgBDNF mice lacking IL-1R1 compared to those with intact IL-1R1. Findings from this study support the role of IL-1R1 signaling in seizure-induced neuronal injury/death in brainstem-cortical networks.

Disclosures: T. Dillon: None. **A. Silverstein:** None. **C. Isgor:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.09/C56

Topic: B.08. Epilepsy

Support: NIH: RO1 NS108808

Title: Advancing Precision Therapies for Dravet Syndrome with Collaborative Cross Mouse Models

Authors: *J. LI[1], Z. SHAKKOUR[1], B. YU[1], J. ZHOU[1], D.-P. LI[2], P. R. CARNEY[3]; [2]Med., [1]Univ. of Missouri, Columbia, MO; [3]Child Hlth., Univ. of Missouri, Columbia, Columbia, MO

Abstract: Objective: Dravet syndrome (DS) exhibits significant heterogeneity in clinical presentations and underlying pathophysiology, resulting in distinct disease subtypes. Individuals with *SCN1A*-associated DS often experience persistent seizures and progressive neurological impairments despite aggressive anticonvulsant therapy. This variability highlights the need for preclinical models that capture the broader spectrum of DS subtypes to enable the development of precision therapeutic approaches. **Methods:** To address limitations in existing preclinical models, Collaborative Cross (CC) mouse strains were obtained from the UNC Systems Genetics Core Facility to introduce genetic diversity. Male 129.*Scn1a*+/− mice were bred with female CC mice to generate F1 progeny with null mutations, while wild-type controls (129.*Scn1a*+/+)

served as comparators. Genotyping confirmed *Scn1a* and *Gabra2* variants. Phenotypic assessments included hyperthermia-induced seizure assays, exploratory activity, motor coordination, and anxiety-like behaviors. Environmental factors such as temperature and humidity were monitored to evaluate their influence on seizure traits. Heritability analyses assessed the genetic background's impact on seizure threshold, duration, and severity. Data were analyzed using logistic regression, non-parametric tests, and clustering in R software. **Results:** Two CC strains, CC011 and CC045, exhibited severe DS-like phenotypes characterized by heightened seizure susceptibility, frequent spontaneous seizures, and premature mortality, with approximately 50% of mice succumbing before study completion. Strain-specific variations in seizure susceptibility mirrored the clinical heterogeneity observed in DS patients. A B6J-specific *Gabra2* variant further underscored the interplay between genetic, environmental, and sex-related factors in DS phenotypes. **Significance:** These findings demonstrate that CC strains effectively model the genetic diversity and clinical variability of human DS. Their utility in identifying genetic modifiers supports the development of precision therapies tailored to specific DS subtypes, advancing translational research and therapeutic discovery.

Disclosures: J. Li: None. **Z. Shakkour:** None. **B. Yu:** None. **J. Zhou:** None. **D. Li:** None. **P.R. Carney:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.10/C57

Topic: B.08. Epilepsy

Title: Olig2-specific loss-of-function Slc35a2 results in hypomyelination and spontaneous seizures

Authors: *T. BARTEL[1], C. GHOSH[3], K. BISAHA[2], J. KHOURY[4], A. NEMES-BARAN[5], I. BLUMCKE[6], I. NAJM[4];
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Abstract: Malformations of cortical development represent major causes of drug-resistant epilepsy, with mild malformation of cortical development with oligodendroglial hyperplasia and epilepsy recently recognized as a distinct pathological entity. Somatic mutations in *SLC35A2*, encoding the uridine diphosphate-galactose transporter, have been implicated in this condition. In this study, we investigated the behavioral, electrophysiological, and cellular/molecular phenotypes associated with loss-of-function *Slc35a2* mutations targeted to oligodendrocytes. A conditional knockout mouse model was generated using an Olig2-specific promoter. Behavioral assessments included neurological scoring, open field, locomotor habituation, and pentylenetetrazole-induced seizure testing in 85 mice (26 female controls, 17 female cKO, 26

male controls, 16 male cKO), aged 4-18 weeks. Continuous EEG studies were performed in 8 control and 8 cKO mice implanted with cortical electrodes. Histopathological analyses and immunohistochemistry were performed at postnatal days 15, 21, and 60. Loss-of-function *Slc35a2* mice exhibited significantly reduced body weight, progressive motor dysfunction, and reduced survival compared to controls. Video-EEG monitoring captured spontaneous electro-clinical seizures in cKO mice, accompanied by frequent interictal spiking, despite preserved neuronal integrity. Histological evaluation revealed extensive subcortical hypomyelination, reactive gliosis, and a significant reduction of Olig2-positive oligodendrocytes in the corpus. Transmission electron microscopy confirmed hypomyelination in genu of corpus callosum. Our results demonstrate that loss of *SLC35A2* function in oligodendrocytes alone is sufficient to reproduce key pathological and clinical features observed in human mild malformation of cortical development with oligodendroglial hyperplasia and epilepsy. Notably, the emergence of spontaneous seizures in the absence of direct neuronal genetic abnormalities highlights a central role for oligodendrocyte dysfunction and hypomyelination in epileptogenesis. These significant findings challenge the traditional neuron-centric view of epilepsy and suggest that targeting glial dysfunction may offer novel therapeutic opportunities.

Disclosures: T. Bartel: None. **C. Ghosh:** None. **K. Bisaha:** None. **J. Khoury:** None. **A. Nemes-Baran:** None. **I. Blumcke:** None. **I. Najm:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.11/C58

Topic: B.08. Epilepsy

Support: PHDF-21-1592

Title: The kainic acid induced status epilepticus. Comparative study of the hippocampus ultrastructure in wistar rats

Authors: *I. SHARIKADZE[1], M. ZHVANIA[1,2], N. JAPARIDZE[2,3], E. GASIMOV[4];
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Abstract: The post status epilepticus (SE) model is one of the most helpful animal models for epilepsy research. In many cases it closely mimics the clinical signs of temporal lobe epilepsy in humans, the most common type of epilepsy in adults. Although behavioral, electrophysiological and molecular alterations following SE have been extensively studied, corresponding structural changes remain less well explored. However, to optimize the treatment of epilepsy and complications of SE, it is essential to know how effectively they prevent seizure-induced neuronal damage. In the present study, we elucidate ultrastructural changes and quantitative

alterations in synaptic parameters in the hippocampal CA1 and CA3 areas of adult male Wistar rats, provoked by kainic acid (KA) induced SE. This drug was injected intraperitoneal, until convulsive seizures were developed. Ultrastructural analysis was performed on 1, 7 and 21 days after SE. During all this period the behavior of animals was monitored. Structural alterations were more numerous in the CA1 area. In general, ultrastructural modifications observed are found to be dependent on the frequency of seizure activities. More extensive alterations were produced by KA after 7 and 21 days SE which induced apoptosis, cell shrinkage, activation of glia, changes in some presynaptic terminals and enlarged astrocyte ensheathment around synaptic components. Morphometric analysis of several parameters of axo-dendritic synapses revealed significant changes in the number of presynaptic terminals, presynaptic mitochondria and length of active zone. Such reflection of epilepsy pathophysiology indicates a relatively long-lasting character of SE-induced ultrastructural alterations. This research was supported by Shota Rustaveli National Science Foundation of Georgia, grant #PHDF-21-1592.

Disclosures: I. Sharikadze: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); This research was supported by Shota Rustaveli National Science Foundation of Georgia, grant #PHDF-21-1592.. **M. Zhvania:** None. **N. Japaridze:** None. **E. Gasimov:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.12/C59

Topic: B.08. Epilepsy

Support: NC 123240.1

Title: Effects of microinjection of ketamine in the thalamic reticular nucleus on PTZ-induced seizures

Authors: *V. M. MAGDALENO-MADRIGAL;
Inst. Nacional De Psiquiatría Ramón de la Fuente Muñiz, Ciudad De México, Mexico

Abstract: The TRN contains inhibitory neurons that release GABA, which generates and controls spike-wave (SWD) and sleep spindles. The goal of this study was to analyze the behavioral and electrographic changes provoked by microinjection of ketamine in the TRN on PTZ-induced seizures. Adult male Wistar rats were used. A tripolar electrode, a cannula guide in the left TRN, and a nail shape in the motor cortex were implanted. The animals were randomly assigned to four groups: SS group, which received a microinjection of saline solution (SS) and PTZ; Ket40 group, which received a microinjection of ketamine (40 ng) and PTZ; Ket60 group, which received a microinjection of ketamine (60 ng) and PTZ; finally, Ket80 group, received a microinjection of ketamine (80 ng) and PTZ. Vehicle and ketamine at a rate of 250nL/1m were injected. We analyzed the SWD number and latency. In addition, the GTCS number, duration,

latency, and severity. Ketamine in all groups decreased the number of spike-wave discharges and increased the seizure severity. A decrease in the power of theta frequency was also found compared to the SS group. In multiunit rerecording of 42 extracellular groups, we observed a firing rate frequency increase (55%), a decrease (19%), and 26 % no changes. Our results suggest that the ketamine in the TRN may have a proconvulsive effect, reducing the survival rate. This effect may be associated with the firing pattern changes in extracellular activity.

Disclosures: V.M. Magdaleno-Madrigal: None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.13/C60

Topic: B.08. Epilepsy

Support: NINDS R25NS130964
NIH Contract: 75N95022C00007

Title: Traumatic brain injury in mice leads to pathologically altered neuronal excitability in the cortex

Authors: *C. MCEVOY, K. S. WILCOX, P. J. WEST;
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Abstract: Post-traumatic epilepsy (PTE) develops in up to one-third of patients who experience a severe traumatic brain injury (TBI). Certain populations (e.g., military personnel and contact sport athletes) are at a higher-risk of experiencing a severe TBI, and the PTE that follows frequently develops into drug-resistant epilepsy. This drug-resistance makes it especially difficult to prevent or modify the development of PTE in at-risk patients. To address this gap in the PTE field, our goal is to establish a reliable, moderate-throughput assay using an in-vitro acute slice electrophysiology mouse model of TBI that has predictive validity for the identification of novel therapies that may impact epileptogenesis in this patient population. Adult CD1 mice are randomly assigned to one of three groups: (1) a controlled cortical impact (CCI) group, (2) a sham surgery (craniotomy without impact) group, or (3) a completely naive control group. Following a 3-week recovery period, ipsilateral and contralateral cortical brain slices (350 μm) adjacent to the injury site are prepared and maintained in warm (30°C) oxygenated artificial cerebrospinal fluid (ACSF) for recordings of stimulation-evoked extracellular field excitatory postsynaptic potentials (fEPSPs). Using a stimulus-response protocol with incrementally increasing stimulus intensities, input-output (I/O) curves are generated from cortical layers underneath or lateral to the injury site. We hypothesize that there will be significant differences in synaptic and/or intrinsic excitability in neurons proximal to the injury in animals who have experienced a CCI. Accordingly, we compare the I/O curves between our CCI, sham control, and naive control groups, and initial experiments suggest changes in the excitability of the cortical

tissue surrounding the injury site consistent with epileptogenic phenotypes. Future studies will further examine the changes in cortical excitability in our CCI mice as well as changes in hippocampal excitability using the same input/output protocol. Furthermore, ongoing studies aim to correlate the physiological changes we are seeing with immunohistochemical markers of gliosis and neuronal loss. Finally, we aim to evaluate the sensitivity of this assay to known and investigational compounds to assess their acute anti-seizure and/or chronic anti-epileptogenic effects. We believe that this assay has the potential to accelerate the early identification of disease-modifying and/or anti-epileptogenic therapies for patients at risk of developing post-traumatic epilepsy.

Disclosures: C. McEvoy: None. **K.S. Wilcox:** None. **P.J. West:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.14/C61

Topic: B.08. Epilepsy

Support: Johannes Dichgans doctoral schoolarship for medical students

Title: Pathophysiological mechanisms of epileptic encephalopathy in the cortex of a dravet syndrome mouse model

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Abstract: Dravet syndrome (DS) is a severe pharmaco-resistant developmental epileptic encephalopathy (DEE) characterized by early-onset epileptic seizures, intellectual disability, and other neurological symptoms. It is mainly caused by de-novo loss-of-function (LOF) variants in the *SCN1A* gene (encoding the voltage-gated Na^+-channel $Na_V1.1$), associated parvalbumin interneuron (PvIN) dysfunction and consecutive disinhibition of neuronal networks.To get a deeper insight into cortical disease pathogenesis, particularly into potential epileptogenic and secondary mechanisms beyond the initial inborn genetic defect, we performed single-cell-resolved transcriptomic, patch-clamp and immunohistochemical analysis of somatosensory cortex (SSC) in a DS mouse model carrying the human recurrent missense variant p.Ala1783Val. Despite Scn1a being critically involved in shaping PvIN action potential firing properties, i.e. conveying fast-spiking characteristics, we only found subtle alterations of somatic excitability of cortical PvINs in Dravet mice around seizure onset. Unexpectedly, electrophysiological recordings revealed emerging intrinsic hyperexcitability of cortical layer V pyramidal cells

(PCs). Concomitantly various voltage-gated potassium ion channel genes were found dysregulated in layer V PCs on transcriptomic level. Small molecule-mediated channel modulation was able to restore physiological level excitability of PCs, thereby supporting a key role of potassium channel dysregulation in evolving excitatory/inhibitory network imbalance in Dravet mice.Similarly, recent analysis of Ca1 hippocampus had demonstrated only mildly altered action potential firing of PvINs. Nevertheless, inhibitory synaptic transmission was strongly reduced in Ca1 and was associated with distal axonal and (pre-)synaptic dysfunction, altered inhibitory synapse numbers and ultrastructural changes (bioRxiv 2023.10.04.560735). We are currently investigating inhibitory synaptic transmission, perform synapse quantifications and ultrastructural synaptic characterization in cortex to understand how *Scn1a* LOF (predominantly in PVIN) mechanistically links to transcriptome dysregulation and hyperexcitability of PCs.

Disclosures: E. Erharhaghen: None. **S. Iavarone:** None. **N. Layer:** None. **U.B. Hedrich:** None. **T.V. Wuttke:** None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.15/C62

Topic: B.08. Epilepsy

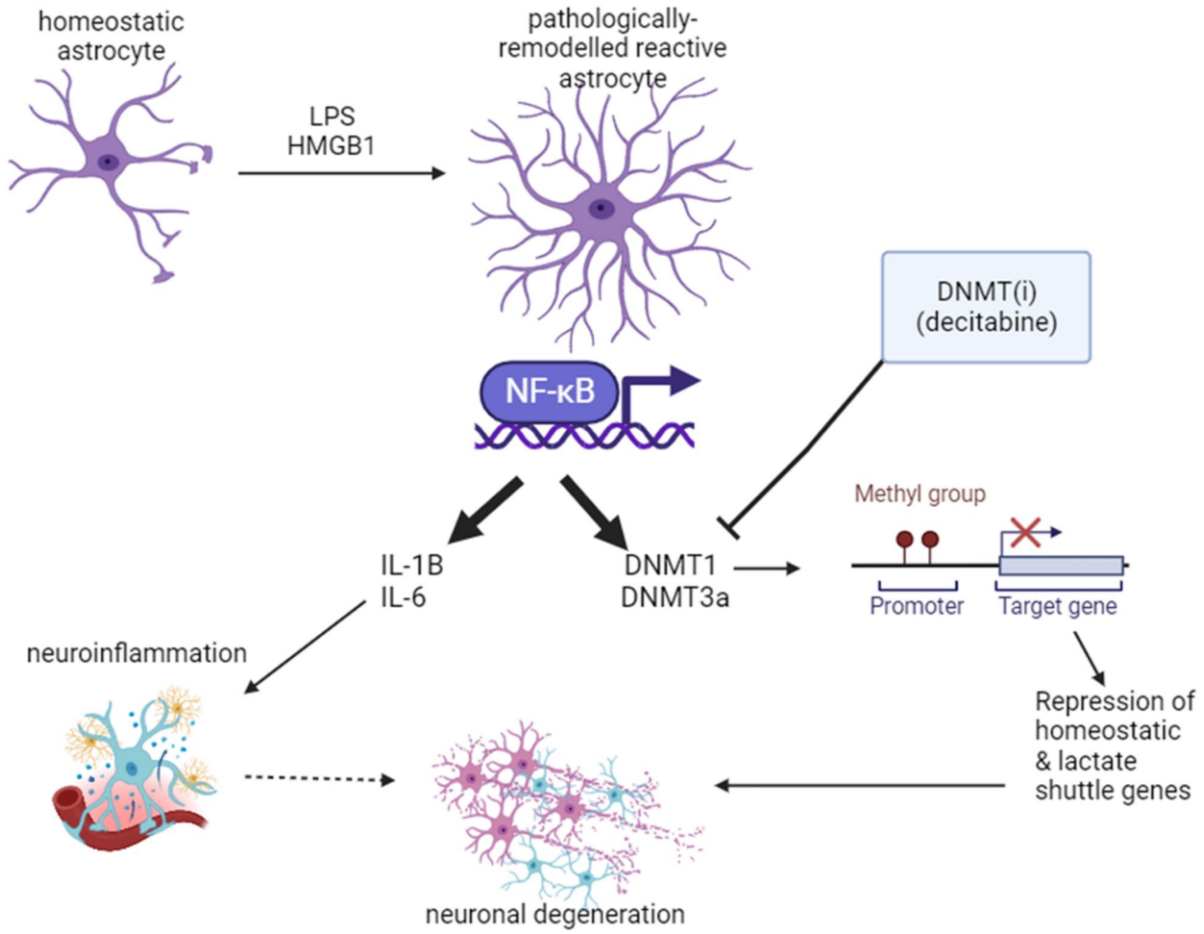
Support: ISM Abstract Slot for the SfN Annual Meeting,

Title: Persistent Epigenetic Alterations in Astrocytes as a Key Mechanism in Temporal Lobe Epileptogenesis

Authors: *J. GOMEZ CUAUTLE;
LIN, Univ. Andina del Cusco, Cusco, Peru

Abstract: Temporal lobe epilepsy (TLE) is the most prevalent form of epilepsy in humans. Retrospective analyses of TLE patients have identified an initial precipitating event (IPE) during early childhood, followed by a clinically silent period during which epileptogenesis takes place, ultimately leading to chronic epilepsy. We hypothesized that epigenetic mechanisms may contribute to epileptogenesis, specifically through astrocytic dysfunction. To investigate this hypothesis, we employed a multi-model approach comprising (1) the lithium-pilocarpine model of TLE in Wistar rats, (2) primary astroglial cultures, and (3) surgically resected hippocampal tissue from TLE patients. In patient samples, we observed reactive astrogliosis, increased DNA methylation, and downregulation of the homeostatic astroglial genes *Kir4.1* and *AQP4* as revealed by immunohistochemistry. In rats subjected to IPE via lithium-pilocarpine (30 mg/kg, i.p.), astrocytes exhibited persistent DNA hypermethylation at 7, 21, and 35 days post-IPE, accompanied by the downregulation of *AQP4*, *Kir4.1*, and *glutamine synthetase (GS)*, as well as upregulation of inflammatory markers (*C3*, *MAFG*) and DNA methyltransferases (DNMTs), as

quantified by qPCR. In primary astrocyte cultures, exposure to the damage-associated molecular pattern (DAMP) HMGB1 (500 ng/ml, 18 h) recapitulated the astrocytic molecular signature observed in vivo. These effects were reversed by treatment with the DNMT inhibitor decitabine (100 µM), indicating a causal role of DNA methylation in astrocytic dysfunction. Collectively, our findings demonstrate that astrocytes undergo pathological epigenetic remodeling following epileptogenic insults, potentially sustaining long-term molecular alterations that contribute to the development and maintenance of epilepsy.



Disclosures: J. gomez cuautle: None.

Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.16/C63

Topic: B.08. Epilepsy

Support: DGAPA-PAPIIT-UNAM IN224523-HLM
CONACYT FC1782-LC
CONAHCYT 2052610-LVM

Title: Streptozocin-induced diabetes mellitus facilitates longitudinal changes in gray and white matter microstructure, and increased susceptibility to status epilepticus in rats

Authors: *L. VAZQUEZ[1], F. GUZMÁN GÓMEZ[2], D. GASCA-MARTINEZ[3], E. DE LOS RÍOS[4], L. CONCHA[5], L. LOPEZ-MERAZ[6], H. LUNA-MUNGUIA[7];
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Abstract: Type 2 Diabetes Mellitus (T2DM) is characterized by chronic hyperglycemia and it has been associated to neurological impairments. Previous studies using diffusion tensor imaging have found microstructural changes in the patient's brains. However, the timing of these abnormalities and how they may contribute to facilitate seizures or *status epilepticus* is still unclear. Three-day-old Sprague-Dawley rat pups were divided in two groups: Control (vehicle sc injection; 8 males and 8 females) and streptozocin (STZ) (75mg/kg sc injection; 20 males and 20 females). Glucose levels were tested with a glucometer every month from birth until 4 months old. All animals were also scanned using a 7T MRI scanner at the same time points. Finally, all the animals received 3-mercaptopropionic acid (MP) every 12 h for 5 days to induce recurrent generalized seizures. Control animals showed blood glucose levels between 90 and 110 mg/dl. However, 11 male and 8 female STZ rats showed levels higher than 140mg/dl since the second collection, remaining elevated until the last one. Interestingly, these animals also showed a significant mean diffusivity increase in dorso-medial thalamus (2nd scan, $p<0.05$), ventral hippocampus (3rd scan, $p<0.05$), and cingulate (3rd scan, $p<0.05$). Regarding fractional anisotropy, females showed significantly decreased values in internal capsule (3rd scan, $p<0.05$), fimbria (4th scan, $p<0.01$), and corpus callosum (4th scan, $p<0.01$); males only showed a significant decrease in fimbria (3rd scan, $p<0.01$). When submitted to the recurrent severe seizures model, the generalized seizures from both sexes tended to evolve to *status epilepticus* since the second MP administration. This induction was considered a sign of high seizure severity. In part, possibly explained by the hippocampal changes (neuronal loss, gliosis) and the reduced FA of certain brain regions (likely the result of axonal loss). Acknowledgements. Imaging was performed at the National Laboratory for MRI. We thank Juan Ortiz and Mirelta Regalado for technical assistance. Funding. DGAPA-PAPIIT-UNAM IN224523-HLM, CONACYT FC1782-LC, CONAHCYT scholarship 2052610-LVM.

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Poster

PSTR377: Mechanistic Studies of Epilepsy in Animal Models II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.17/C64

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

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National Research Foundation (NRF) funded by the Korean government (MSIT) (RS-2023-00220408)
National Research Foundation of Korea(NRF) funded by the Ministry of Education (RS-2023-00248384)

Title: Photobiomodulatiuon Attenuates Secondary Olfactory Dysfunction and Promotes Functional Recovery in an Animal Model of Status Epilepticus

Authors: *H. GOO[1], N. HONG[2], J.-C. AHN[2];
[1]Dankook Univ., Choenan-si, Korea, Republic of; [2]Dankook Univ., Cheonan-si, Korea, Republic of

Abstract: Photobiomodulation (PBM) is a non-invasive therapeutic approach that uses red or near-infrared light and has demonstrated beneficial effects in both central and peripheral nervous system disorders. Status epilepticus (SE) is a life-threatening neurological condition caused by prolonged abnormal electrical activity, often resulting in widespread neuronal loss and neuroinflammation. Given the close anatomical and functional connections between the olfactory system and other brain regions, SE can lead to secondary olfactory dysfunction, a symptom that varies across individuals and remains underexplored. In this study, SE was induced in 7-week-old C57BL/6 mice via administration of scopolamine and pilocarpine. Following SE induction, 830 nm PBM was applied transcranially. Olfactory function was evaluated using a food-finding behavioral test. One week post-induction, brains and nasal tissues were harvested and analyzed through histological staining. Behavioral assessments revealed impaired olfactory function after SE. Histological analysis showed increased expression of glial fibrillary acidic protein (GFAP) in the hippocampus and elevated c-Fos expression in the olfactory cortex, indicating neuroinflammatory and hyperactivity responses. These changes were significantly reduced in the PBM-treated group. Additionally, structural alterations in the olfactory epithelium and reduced expression of olfactory marker protein (OMP) in nasal tissues were observed in SE mice, both of which were ameliorated by PBM treatment. These findings suggest that PBM can mitigate SE-induced secondary olfactory dysfunction and support its therapeutic potential for functional recovery. This study also lays the groundwork for future mechanistic investigations into PBM-mediated neuroprotection.

Disclosures: H. Goo: None. **N. Hong:** None. **J. Ahn:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.01/D1

Topic: B.10. Neuro-Oncology

Support: NIH/NCI GRANT R01 CA269365

Title: New insight into regulation of mitochondria transfer in the glioma microenvironment.

Authors: *N. FILIPPOVA, H. NAMKOONG, J. SHI, L. NABORS;
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Abstract: Neutrophils are recognized as highly heterogeneous with strong plasticity toward immunosuppressive immature populations in aging and cancer. The myeloid-derived microenvironment is mostly represented by immunosuppressive TREM1^{+}- neutrophils in mouse GBM models. Using a transgenic mouse model with fluorescently labeled neutrophils and mitochondria belonging to neutrophils, we identified neutrophils as a source of horizontal mitochondria transfer. In vitro, experiments confirmed mitochondria transfer from primary mouse neutrophils and neutrophil-like differentiated HL60 cells to mouse GBM and PDGx cell lines, respectively. Using neutrophils with RFP^{+}-labeled mitochondria and a PDGx cell line expressing green fluorescence protein (EGFP^{+}), we performed a CRISPR genome-wide knockdown screening to select PDGx cells unable to accept mitochondria from neutrophils. GBM cells acquiring mitochondria (EGFP^{+}RFP^{+}) were separated from GBM cells unable to acquire mitochondria (EGFP^{+}RFP^{-}) by flow cytometry. The EGFP^{+}RFP^{-} population comprised less than 1% of the total cell library and was less aggressive *in vivo* with overall mouse survival of around 50 days compared to 26 days for mice with intracranial tumors formed by the original cell library and the RFP^{+}EGFP^{+} population. *In vitro*, the EGFP^{+}RFP^{-} and RFP^{+}EGFP^{+} populations exhibited a similar rate of proliferation. However, a striking difference was observed in cell migration patterns and tumor morphology. Intracranial tumors formed with the EGFP^{+}RFP^{-} population exhibited smooth well-defined borders while tumors formed with the EGFP^{+}RFP^{+} population had irregular jagged borders with patches of migrating cells in the adjusted tissue. Genomic DNA were isolated from both pools of cells, and amplicons corresponding to gRNAs were generated and sequenced. Amplicon analysis revealed more than 1000-fold enrichment of GBM cells with gRNA-guided CCDC144NL-AS1 gene knockdown in the EGFP^{+}RFP^{-} population. On the protein level, the EGFP^{+}RFP^{-} population exhibited a significant enhancement in the expression of CDH1 and YNF185 proteins and a slight decrease in VIM1. Immunostaining confirmed strong CDH1 cell-surface expression tightening cell-to-cell contacts between GBM cells in EGFP^{+}RFP^{-} population *in vitro* and *in vivo*. Our data conclude that i) neutrophils infiltrate the GBM microenvironment, ii) neutrophils could be the source of mitochondria transfer to GBM cells, iii) neutrophil-guided mitochondria transfer is associated with GBM progression and migration iv) CDH1 overexpression in GBM cells is associated with reduction of tumor spread and ability of mitochondria hijacking from microenvironment.

Disclosures: N. Filippova: None. **H. Namkoong:** None. **J. Shi:** None. **L. Nabors:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR378.02/D2

Topic: B.10. Neuro-Oncology

Support: NIH 1P01CA245705-01A1

Title: Tumor-associated macrophages protect glioblastoma cells from ferroptotic stress in a sex-biased manner by inducing ferritin-bound iron release from cancer cells via exosomes

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Abstract: Glioblastoma (GBM) is a highly aggressive and difficult-to-treat brain tumor originating from astrocytes. It is also sexually dimorphic, with females having a survival advantage and responding better to standard therapy. Sex-biased differences in iron metabolism play a significant role in making GBM a sexually dimorphic disease. Iron is a critical player in tumor metabolism, as cancer cells require iron to keep dividing rapidly; however, high cellular iron can make the cells vulnerable to ferroptosis. Macrophages play a pivotal role in tumorigenesis by regulating iron homeostasis in the tumor microenvironment. Here, we examine macrophage iron homeostasis as a potential driver of sex biases in glioblastoma. Using bone marrow-derived macrophages (BMDMs) from C57BL/6 mice, we found that male macrophages exhibited higher iron uptake (Fe^{57} measurement by ICP-MS), greater iron storage (FTL expression), and a larger labile iron pool (ferro-far red staining) than females. These results indicate that macrophage iron metabolism is sexually dimorphic.
Next, we used a transwell co-culture system to study how macrophages regulate the iron and ferroptotic status of GL261 cells. Upon RSL3 treatment to induce ferroptosis, GL261 cells in coculture with BMDMs had significantly higher cellular viability (Cell Titer Glo) and significantly less lipid peroxidation (Bodipy Staining) than GL261 cells cultured alone, indicating that tumor-associated macrophages confer protection to GBM cells from ferroptotic stress. To further understand the underlying mechanisms, we studied the iron homeostasis in the GL261 cells. Immunoblot analysis showed that GL261 cells cocultured with BMDMs had a significant reduction in cellular FTH1 in a sex-biased manner. This reduction in cellular FTH1 correlated with decreased total cellular iron. Further immunoblot analysis of the conditioned media revealed increased secreted FTH1 levels from GL261 cells in coculture. Fe^{57} loading experiments showed that this increased secreted FTH1 levels correlated with an increased iron secretion, and this FTH1-bound iron secretion acts as a major pathway. Further immunoblot analysis indicated that GL261 cells in coculture with BMDMs had increased CD63 and decreased IRP2 levels, indicating that CD63-positive exosomes might be involved in the FTH1 release. Exosome isolation confirmed enrichment of CD63 and FTH1 protein levels in co-cultured GL261-derived extracellular vesicles. This iron efflux mechanism, independent of Ferroportin and Hepcidin, may serve as a key route by which GBM cells evade ferroptosis during inflammation, with macrophage-driven sex differences playing a regulatory role.

Disclosures: A. Sahu: None. **K. Palsa:** None. **G. Shenoy:** None. **B. Webb:** None. **J.R. Connor:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

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Program #/Poster #: PSTR378.03/D3

Topic: B.10. Neuro-Oncology

Support: Kakenhi Grabt P24K18216B
Kakenhi Grabt P22K06429B

Title: Histone H2B E3 Ubiquitin ligase RNF20 drives glioblastoma stem-like cells maintenance and progression

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Abstract: Glioblastoma multiforme is a highly heterogeneous tumor. Resistance to standard therapies is frequent, suggesting that a small subpopulation of glioblastoma stem-like cells (GSCs) within the tumor causing tumor recurrence. It can be distinguished from the tumor cells through the epigenetic regulatory program and disruption in epigenetic processes governs molecular alteration leads to malignant transformation. Here, we demonstrated that the histone H2B E3 ubiquitin ligase RNF20 is necessary for GSCs maintenance and tumor progression. RNF20 downregulations sustained the GSCs pool at a low-proliferation rate, where it possesses long-term self-renewal and higher tumorigenic potential. Besides, RNF20 knockdown cells are more resistant to Temozolomide and reduce apoptosis. The transition to a proliferative state depended upon RNF20 expression patterns, thereby rendering GSCs dependent on RNF20 to maintain their growth and transcriptional processes. In addition, our data suggest that epigenetic changes and interaction between glioma cells and neurons in the healthy region affect the prognosis of the glioma-bearing patients.

Disclosures: N. Morimura: None. **K.A. Daun:** None. **N. Mohd Ariffin:** None. **K. Nukada:** None. **C. Hayashi:** None. **S. Hitoshi:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

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Program #/Poster #: PSTR378.04/D4

Topic: B.10. Neuro-Oncology

Support: R01 NS127424

Title: Unveiling the role of ST6Gal1 in regulating metabolic shifts in glioblastoma: Insights into GLUT3 dynamics

Authors: *J. SAAD[1], S. GC[6], G. BENAVIDES[2], T. NEMKOV[7], K. TUY[8], J. A. MOBLEY[3], V. DARLEY-USMAR[4], A. D'ALESSANDRO[7], S. BELLIS[5], A. HJELMELAND[5]; [2]Pathology, [3]Anesthesiol., [1]Univ. of Alabama at Birmingham, Birmingham, AL; [4]Pathology, Univ. of Alabama at Birmingham, Birmginahm, AL; [5]Cell, Develop. and Integrative Biol., Univ. of Alabama at Birmingham, Birmingham, AL; [6]Herbert Irving Comprehensive Cancer Ctr., Columbia Univ., New York, NY; [7]Biochem. and Mol. Genet., Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [8]Cell, Develop. and Integrative Biol., Univesity of Alabama at Birmingham, Birmingham, AL

Abstract: Despite the importance of glycosylation in regulating protein folding, modulating cell-cell interaction, and modifying signal transduction, its role has been somewhat overlooked in diseases and malignancies, unlike other post translational modifications like phosphorylation. Beta-galactoside α2,6-sialytransferase 1 (ST6Gal1) is a glycotransferase in the Golgi that adds a terminal α2,6-linked sialic acid (α2,6 sialylation) to glycoproteins, consequently modulating their dynamics and function at the cell surface. ST6Gal1-mediated α2,6 sialylation is likely context dependent, in part, resulting from environmental pressures; yet its role in diseases including brain malignancies has been underexplored. Recently, our lab demonstrated that ST6Gal1 is upregulated in glioblastoma (GBM), the most frequent and aggressive primary brain tumor in adults, and acts as a critical regulator of stemness in a subpopulation of therapy resistant and metabolically plastic GBM brain tumor initiating cells (BTICs). Metabolic reprogramming in BTICs represents one mechanism underlying their therapeutic resistance and survival in the harsh nutrient-deprived tumor microenvironment. Here, we investigate the link between ST6Gal1-mediated α2,6 sialylation and metabolic reprogramming in GBM. Using the Seahorse stress test, we show that low α2,6 sialylation correlates with decreased glycolysis and glycolytic capacity. ST6Gal1 knock down (KD) decreases glucose uptake and promotes metabolic reprogramming, as revealed by untargeted metabolomics of GBM patient derived xenografts. Because α2,6 sialylation by ST6Gal1 can alter conformation, clustering, and retention of glycoproteins including metabolic receptors, we complemented our study with proteomic profiling of α2,6 sialylated proteins and identified neuronal glucose transporter 3 (GLUT3) as a novel target. GLUT3, the predominant glucose transporter in BTICs, has a single N-glycan target for α2,6 sialylation; however, the biological consequence of GLUT3 α2,6 sialylation remains unknown. Using high resolution imaging, we demonstrate that ST6Gal1 KD decreases GLUT3 levels at the cell surface, thereby impairing glucose uptake and glycolysis. Taken together, our findings establish ST6Gal1 as a modulator of GBM metabolic plasticity through α2,6 sialylation of key metabolic receptors, notably GLUT3, uncovering therapeutic vulnerabilities in GBM and potentially other brain diseases where GLUT3 plays a crucial role.

Disclosures: J. Saad: None. **S. Gc:** None. **G. Benavides:** None. **T. Nemkov:** None. **K. Tuy:** None. **J.A. Mobley:** None. **V. Darley-Usmar:** None. **A. D'Alessandro:** None. **S. Bellis:** None. **A. Hjelmeland:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

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Program #/Poster #: PSTR378.05/D5

Topic: B.10. Neuro-Oncology

Title: Deciphering the key mediator behind truncated TrkB receptor, TrkB.T1's, role in morphological control.

Authors: *H. L. JAGANA[1], M. SHABAR[3], L. MERINO GALAN[2], M. HATHAWAY[3], A. RAJENDRAN[4], L. RUBIO[3], N. RECHE-LEY[3], D. E. JOHNSON[2], S. S. PATTWELL[5]; [2]Ben Towne Ctr. for Childhood Cancer Res., [1]Seattle Children's Res. Inst., Seattle, WA; [3]Ben Towne Ctr. for Childhood Cancer Res., Seattle Children's Res. Inst., Seattle, WA; [4]Univ. of Washington Sch. of Med., Seattle, WA; [5]Human Biol., Seattle Children's/University of WA Sch. of Medicine/Fred Hutchinson Cancer Ctr., Seattle, WA

Abstract: The neurotrophin receptor TrkB, along with its ligand brain derived neurotrophic factor (BDNF) are critical for proper neural development. The truncated splice variant, TrkB.T1, is involved in glial development. It contains altered expression in many neurological diseases and is overexpressed across several cancers including low grade glioma and glioblastoma. TrkB.T1 stimulation by BDNF initiates a signaling cascade that increases intracellular Ca^{2+} in astrocytes. In addition, TrkB.T1 associates with Rho-GDI1 leading to morphological changes in astrocytes and glioma cells. Both Ca^{2+} transients and Rho GTPases regulate neural developmental pathways including cytoskeletal changes. Although links between TrkB.T1 and Ca^{2+} signaling and TrkB.T1 with Rho GTPase activity have been individually identified, a gap in how they interact to maintain TrkB.T1 specific signaling and the implications of such signaling throughout the lifespan remains. We hypothesize that TrkB.T1 specific Ca^{2+} mobilization and Rho activation are linked and together are indicative of TrkB.T1's role in development and dysregulation in neurological disorders and cancers. Our work demonstrates a convergence between these two pathways through morphological control. Patient derived glioma stem cells with high and low expression levels of TrkB.T1 were utilized to investigate their Ca^{2+} and morphological differences. Changes in intracellular Ca^{2+}, fluorescently marked by Fluo-4AM, were observed dependent on TrkB.T1 expression levels. Through pharmacological manipulation, we aimed to understand how TrkB.T1-dependent Ca^{2+} and RhoGTPase levels altered the cytoskeleton. F-actin was stained with phalloidin and visualized using immunofluorescence microscopy. Western blot and co-immunoprecipitation were used for further analyses of downstream signaling and binding partners to clarify the mediators of the observed morphological changes. Our results contribute to the growing body of evidence that TrkB.T1 possesses signaling capabilities beyond its previously characterized dominant-negative function.

This work helps to unveil TrkB.T1's specific role in neurodevelopment and the impact of its dysregulation.

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Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

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Topic: B.10. Neuro-Oncology

Support: Hjärnfonden Scholarship 2024-2026
Sture Erikssons fondation travel grant

Title: Uncovering chromatin state alterations in diffuse intrinsic pontine glioma

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Abstract: Diffuse Intrinsic Pontine Glioma (DIPG) is a highly aggressive pediatric brain tumor that diffusely infiltrates the pons and accounts for approximately 80% of all pediatric brainstem tumors. A hallmark of DIPG is the lysine-to-methionine substitution at position 27 in histone H3 (H3K27M), commonly occurring in genes such as H3F3A and HIST1H3B. This mutation disrupts the normal deposition of H3K27 trimethylation (H3K27me3) not only on the mutant histones but also on wild-type counterparts, leading to a global reduction of H3K27me3. Consequently, there is a compensatory increase in H3K27 acetylation (H3K27ac), resulting in aberrant transcriptional activation. However, the epigenetic basis of cellular heterogeneity in DIPG remains poorly understood.In this study, we applied nanoCT, a single-cell CUT&Tag approach that utilizes nanobody-Tn5 fusions to simultaneously profile H3K27me3 and H3K27ac in the same cell. Given that epigenetic dysregulation is a key driver of DIPG, our multimodal epigenomic analysis provides insights into the chromatin state landscape at single-cell resolution. This work identifies candidate epigenetic regulators and may inform the development of novel therapeutic strategies for this lethal disease.

Disclosures: L. Zhang: None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.07/D7

Topic: B.10. Neuro-Oncology

Title: Tead1 epigenetic silencer curbs glioblastoma malignancy by silencing the yap/taz oncogenic transcriptional cascade

Authors: *E. BARONCHELLI[1], S. FERRETTI[2], A. COVINO[3,4], A. SESSA[4], V. BROCCOLI[4,5];
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Abstract: The molecular implications underlying the relapses of glioblastoma multiforme (GBM), that represent the major challenges to counteract GBM, are not yet well characterized nowadays. Indeed, GBM recurrence is inevitable. Despite current treatments, virtually all patients will relapse due to the presence of cancer stem cells (CSCs) that are able to self-renew and regenerate the tumor, remaining quiescent or exhibiting very low proliferative activity. Considered the urgent medical need to counteract GBM recurrence, we generated a synthetic epigenetic silencing factor called TES (TEAD1 epigenetic silencer) in order to stably switch off the YAP/TAZ oncogenic transcriptional cascade which drives tumor malignancy. TEAD1, in association with YAP and TAZ coactivators, plays a key role in GBM pathogenesis and progression since their interaction initiates the expression of a wide set of genes associated with epithelial-to-mesenchymal transition, cell migration, proliferation and invasion. Notably, TEAD1 binding domain is fused with the KRAB (Kruppel associated box from the Zinc finger protein ZNF10) repressor domain and DNA methyltransferase 3A/L catalytic domains. In this way, TES silences the transcriptional network controlled by TEAD1, the nuclear transducer of the YAP/TAZ signalling pathway, thus curbing GBM malignancy. TES anti-tumoral activity was tested both *in vitro* and *in vivo* GBM models, and its transcriptional output and epigenetic activity was assessed. TES induced a robust transcriptional silencing of a large group of YAP/TAZ targets genes by the induction of stable epigenetic modifications as assessed by RNA-sequencing and RTqPCR. Moreover, TES promoted high cell death and a strong proliferative loss in glioma cell lines (SNB19 and U87) and in patient-derived GBM CSCs *in vitro*. Besides, TES induced a significant reduction of migration in SNB19 as assessed by wound healing assay (n=3 per group). In accordance, local viral delivery of TES in orthotopic intracranial mouse xenograft significantly decreased the development of the human GBM-like masses (-80% ± 9; n=3 per group). Interestingly, the concomitant treatment of Temozolomide (TMZ) with TES increased the overall survival of more than 2 months (Mock vs. TES *p=0.0279, Gehan-Breslow-Wilcoxon test, n=8 per group). Finally, TES administration was assessed not to be toxic for healthy brain cells both *in vitro* and *in vivo* (n=5 per group). Collectively, the strategy exploited by TES consists in silencing the entire transcriptional network controlled by TEAD1 through epigenetic editing, thus achieving high levels of efficacy against GBM recurrences and malignancy.

Disclosures: E. Baronchelli: None. **S. Ferretti:** None. **A. Covino:** None. **A. Sessa:** None. **V. Broccoli:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

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Topic: B.10. Neuro-Oncology

Support: AIRC Grant MFAG 2021

Title: Validation of SOX2 Epigenetic Silencer to reduce glioblastoma in immunocompetent mouse model

Authors: *S. FERRETTI[1], E. BARONCHELLI[3], A. COVINO[4,2], S. MUGGEO[5], V. BROCCOLI[5,6], A. SESSA[5];
[1]Vita Salute San Raffaele Univ., Milano, Italy; [2]Vita Salute San Raffaele Univ., Milan, Italy; [3]Vita-Salute San Raffaele Univ., Milan, Italy; [4]Bicocca Univ., Milano, Italy; [5]San Raffaele Scientific Inst., Milan, Italy; [6]Natl. Res. Council CNR, Milan, Italy

Abstract: Current therapies are still unsatisfactory in preventing recurrences of glioblastoma multiforme (GBM). Tumor-initiating cancer cells infiltrate the parenchymal tissue and become resistant to adjuvant treatments supporting relapses. Studies have shown that SOX2 is frequently upregulated in GBM patient samples and associated with an increase of tumor aggressiveness. Indeed, its inactivation has been attempted with various technologies without achieving complete gene silencing. By rational engineering of SOX2 transcription factor (a key promoter of GBM malignancy) through the assembling with domains from epigenetic repressors (the Kruppel-associated box and DNA methyltransferase3A/L catalytic domains), a synthetic factor called SES (Sox2-Epigenetic-Silencer) has been generated to induce the silencing of the entire pathway regulated by Sox2 itself. Indeed, SES induces gene deregulation in SNB19 2 days after the lentiviral transduction, as assessed by RNA sequencing. Moreover, Gene Ontology analysis indicates that genes associated with apoptosis are up-regulated, whereas genes related with the cell cycle regulation are down-regulated upon SES expression. These results are confirmed *in vitro* on both SNB19 and patient derived Cancer Stem Cells, that showed a strong reduction in cell proliferation and significant increase of cell death 60% (±18) (n=3), after SES lentiviral transduction. Then, *in vivo*, SES local lentiviral delivery in orthotopic xenografts lead to strong regression of tumor and survival rescue, compared with the control group. However, to prove its translational power, its efficacy and safety must be confirmed also in immunocompetent mouse models of GBM. To do so, we are exploring the syngeneic model, trough the injection of Gl261 cells in C57Bl6 mice and evaluating the immune response after SES treatment. Preliminary findings revealed a significant reduction 60% (±11) (n=3) of the tumoral mass in SES treated mice in respect to the control group, confirming its efficacy also in the syngeneic model. Moreover, we are characterizing the immune infiltration during the treatment to confirm, beyond its efficacy, also its safety. Collectively, SES inhibits tumor development by blocking tumoral cell growth in immunodeficient and immunocompetent GBM bearing mice.

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Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

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Support: The Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
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Title: Modeling interaction of glioblastoma, tumor-associated endothelial cells, and neurons in the tumor microenvironment

Authors: *J. PERAZA[1], M. C. CONDRO[2], H. I. KORNBLUM[3];
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Abstract: Glioblastoma (GBM: WHO Grade IV glioma) is a lethal form of brain tumor with a median survival rate of 12 to 18 months. Current treatment strategies, including maximal surgical resections, radiation and chemotherapy with temozolomide have only modestly increased survival times so new therapeutic strategies are desperately needed. The interaction of GBM and the other cell types in the tumor microenvironment play an important role in progression of disease, but the effect of this environment on neurons is poorly understood. It is now well established that neurons synapse onto glioma cells and that this enhances glioma growth. However, the mechanisms by which neurons are induced to form these connections are not entirely understood. Glioma vasculature is known to provide trophic support for glioma cells, but it is unknown whether this vasculature can contribute to neuron growth into the glioma. Here we examine the effect of tumor endothelial cell (TEC) conditioned media on neuronal viability and neurite outgrowth using an in vitro model of cortically patterned NGN2-induced neurons derived from human embryonic stem cells. Surprisingly, our data suggest that exposure to TEC conditioned media inhibits neuronal survival and neurite outgrowth. This research emphasizes the need for a deeper understanding of tumor-neuron interaction pathways in the glioma microenvironment which can ultimately lead to the development of strategic therapies manipulating the neuron-glioma crosstalk to both slow tumor progression and improve neuronal survival, repair, and function.

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Poster

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Program #/Poster #: PSTR378.10/D10

Topic: B.10. Neuro-Oncology

Title: Microbiota-guided modulation of tumor gene expression and survival in glioblastoma: evidence from integrated human and murine model analyses.

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Abstract: The gut microbiome is increasingly recognized as a critical regulator of cancer progression through the gut-brain axis, influencing tumor biology via immune, metabolic, and neuroendocrine pathways. However, its impact on tumor gene expression and survival outcomes in glioblastoma (GBM) remains insufficiently understood. To investigate this relationship, stool and tumor samples were collected from newly diagnosed IDH-wild type GBM patients. Gut microbiota composition was analyzed using 16S rRNA sequencing and processed with the ATHIMA2 pipeline, while tumor RNA was profiled using the NanoString platform. Microbial taxa were correlated with tumor gene expression through Spearman correlation and functional enrichment analyses (KEGG/GO). Linear discriminant analysis (LEfSE) identified bacterial taxa that distinguished long-term (LTS) and short-term survivors (STS) in a subset of 18 patients. Survival associations were further validated in an expanded cohort (n=48) using univariate and multivariate models. To assess therapeutic relevance, we used a murine GBM model in which mice received either a defined mixture of beneficial bacteria or fecal microbiota transplantation (FMT) via oral gavage, and survival was compared across treatment groups. In GBM patients, LEfSE analysis revealed that Lachnospiraceae, Lachnospirales, and Butyricicoccaceae were elevated in STS. Lower Lachnospirales abundance was associated with downregulation of tumor-promoting genes, including GLUD1, CBR4, and NEU1, correlating with improved survival (20.3 vs. 11.8 months). Conversely, high Lachnospiraceae abundance is linked to enhanced tryptophan and purine metabolism, NK cell dysregulation, and increased production of immunosuppressive metabolites such as kynurenine, contributing to a tumor-promoting microenvironment. In the murine model, FMT-supplemented mice demonstrated a significant improvement in survival compared to controls, supporting the therapeutic relevance of microbiome modulation. These findings highlight a previously underappreciated relationship between gut microbiota and GBM progression, revealing that microbial composition significantly influences tumor gene expression, immune dynamics, and clinical outcomes. Functional validation in vivo further supports the potential of microbiota-targeted strategies, such as FMT and probiotic supplementation, as promising adjuncts in glioblastoma therapy.

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Poster

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Topic: B.10. Neuro-Oncology

Support: 1S05EU000046

Title: Combining 3D imaging with light sheet guided 2D multiplex analysis in a glioblastoma mouse model

Authors: *K. BIGOTT[1], V. SCHOPPEL[2], M.-C. TIVERON[5], H. CREMER[6], S. MERZ[2], M. JUNGBLUT[3], A. BOSIO[4];
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Abstract: Spatial biology is an emerging field that studies the spatial organization of cells and molecules within tissues to better understand complex biological processes. Currently available methods may only provide information from thin tissue sections, limiting analysis to rather roughly selected areas, while sectioning is time consuming and often prone to artifacts, especially if slicing entire samples. Our workflow combines 3D imaging with light sheet guided multiplex spatial analysis to get an overview of complex organs, identify target structures within and to further analyze a carefully selected region in depth with multiparameter 2D microscopy - all on the same valuable specimen. While clearing methods in combination with light sheet fluorescence microscopy have emerged as powerful tools for 3D reconstruction of tissues, most clearing techniques involve toxic reagents which require special safety provisions and generate extra effort for sample preparation as well as imaging. To address these shortcomings, we developed an optimized clearing workflow based on non-toxic reagents that allows for efficient 3D imaging (MACS® Deep Clearing Kit) of even whole organs. We performed staining, clearing and 3D light sheet imaging of mouse brain hemispheres derived from a glioblastoma mouse model using the MACS® Deep Clearing Kit and the UltraMicroscope Blaze™. With the full specimen's 3D view, we were able to identify GFP positive glioblastoma cells using an anti-GFP antibody in order to prepare tissue sections that precisely cover these regions of interest, a process termed light sheet guided sectioning for 2D immunofluorescence analysis. The tissue sections were further analyzed by MACSima™ Imaging Cyclic Staining (MICS) technology, providing deep spatial protein phenotyping of individual cells on a single sample. Remarkably, the fluorochrome conjugate used for 3D-IF staining remained detectable after sectioning, enabling us to verify the location of the target region. Furthermore, most epitopes remained stable throughout the entire process of sample preparation for 3D imaging, 3D imaging itself, and sample preparation for 2D microscopy, allowing for deep phenotyping via MICS using relevant glioblastoma, neural cell and immune cell markers. Thus, we have demonstrated that it is possible to apply the MICS technology to a previously cleared tissue, providing a workflow that combines two cutting edge imaging technologies. The ability to obtain both, a comprehensive 3D context, and detailed information about cellular diversity in a spatial context by multiparameter 2D microscopy from a single sample opens up new possibilities in spatial biology.

Disclosures: K. Bigott: A. Employment/Salary (full or part-time): Miltenyi Biotec B.V. & Co. KG. **V. Schoppel:** A. Employment/Salary (full or part-time): Miltenyi Biotec B.V. & Co. KG. **M. Tiveron:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); IBDM-CNRS-Aix Marseille University. **H. Cremer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); IBDM-CNRS-Aix Marseille University. **S. Merz:** A. Employment/Salary (full or part-time): Miltenyi Biotec B.V. & Co. KG. **M. Jungblut:** A. Employment/Salary (full or part-time): Miltenyi Biotec B.V. & Co. KG. **A. Bosio:** A. Employment/Salary (full or part-time): Miltenyi Biotec B.V. & Co. KG.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.12/D12

Topic: B.10. Neuro-Oncology

Title: Uncovering Spatial Proteomic Signatures in Neurodegeneration and Brain Tumors Using Akoya Biosciences' Human Neurobiology Workflow and ariadne.ai SPATIAL

Authors: Y. PEYMANFAR[1], M. POULIN[1], L. SCHUETZ[2], ***F. SVARA**[2], R. MIHANI[1]; [1]Akoya Biosci., Marlborough, MA; [2]ariadne.ai ag, Buchrain, Switzerland

Abstract: Deciphering the spatial proteomic landscape of the human brain is critical for understanding the cellular complexity underlying neurodegenerative disorders and brain tumors. Here, we present an integrated spatial proteomics workflow leveraging Akoya Biosciences' PhenoCode™ Human Neurobiology Panel and PhenoCycler®-Fusion 2.0 platform, combined with ariadne.ai's spatial analysis suite, SPATIAL™.
FFPE brain tissue from human control, Alzheimer's disease (AD), and glioblastoma donors were stained with the 42-plex neurobiology panel and imaged using the PhenoCycler®-Fusion 2.0 system. This high-throughput platform enables multi-slide imaging at single-cell resolution while preserving fragile tissue architecture through its isothermal, barcode-based chemistry.
Spatial analysis was performed using ariadne.ai's platform, which enables flexible, user-driven phenotyping with customizable marker hierarchies. SPATIAL was used to obtain a detailed segmentation of neurons, glia and pathological aggregation of phospho-Tau and beta Amyloid, enabling immune landscapes and neuroinflammatory responses to be spatially mapped and related to hallmarks of AD pathology.
This end-to-end spatial proteomics workflow provides researchers with a scalable and customizable solution to interrogate cellular architecture and pathology in complex brain tissue. It offers a powerful framework for uncovering disease-specific cellular states and mapping functional tissue organization in both neurodegenerative disease and brain oncology research.

Disclosures: Y. Peymanfar: A. Employment/Salary (full or part-time): Akoya Biosciences. **M. Poulin:** A. Employment/Salary (full or part-time): Akoya Biosciences. **L. Schuetz:** A. Employment/Salary (full or part-time): ariadne.ai ag. E. Ownership Interest (stock, stock options,

royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ariadne.ai ag. **F. Svara:** A. Employment/Salary (full or part-time): ariadne.ai ag. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ariadne.ai ag. **R. Mihani:** A. Employment/Salary (full or part-time): Akoya Biosciences.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.13/D13

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Title: Spatial proteomic profiling of glioblastoma and normal human brain microenvironments using high plex digital spatial analysis

Authors: *C. LEE[1], A. BAHRAMI[6], M. VANDENBERG[8], M. MCKENNA[1], H. ZHAI[1], S. HE[1], J. BEECHEM[7], **P. DIVAKAR**[2,3], J. LYSSAND[4], A. HECK[5], K. YOUNG[5];
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Abstract: Glioblastoma (GBM) progression involves significant spatial heterogeneity across tumor and adjacent normal tissue compartments, with regional differences in immune signaling, angiogenesis, and cell state transitions. While bulk or single-cell methods provide useful molecular snapshots, they often fail to preserve spatial context—an essential aspect of understanding the tumor microenvironment (TME). To address this, we applied the Bruker GeoMx® Digital Spatial Profiler (DSP) and the Neural Proteome Atlas (NPA), comprising over 500 protein targets, to formalin-fixed paraffin-embedded (FFPE) sections of human GBM tissues. We specifically selected regions that locate between normal tissue area and invasive tumor area, and studied the proteomic and transcriptomic profiles to analyze the microenvironment of cancer metastasis. Using immunofluorescence-based region selection, we segmented and profiled tumor cores, invasive margins, and peritumoral stroma, achieving high-resolution spatial proteomic maps with deep coverage of neuroinflammatory, angiogenic, and immunosuppressive pathways. Analysis of these defined regions revealed marked enrichment of markers associated with mesenchymal transition (e.g., VIM, CD44), T cell exhaustion (e.g., PD-1, LAG3), and pro-angiogenic signaling (e.g., VEGFR2) in GBM-infiltrated zones. In contrast, normal brain regions maintained homeostatic expression of neuronal (NEFL), astrocytic (GFAP), and vascular integrity markers. The NPA further enabled cell type classification across microanatomical regions, and spatial proteomic signatures distinguished between immunologically active and suppressed niches. Our use of regionally-defined differential protein expression highlighted the ability of DSP to resolve fine-scale variation in tumor-associated signaling networks, including PI3K/AKT/mTOR, MAPK, and immune checkpoint pathways.

Together, these results demonstrate the power of GeoMx DSP high-plex spatial proteomics to resolve region-specific signaling patterns in brain tumors. By integrating protein measurements with spatial precision, this approach offers an analytically applicable framework for biomarker discovery, disease stratification, and evaluating biomedical interventions in glioblastoma.

Disclosures: C. Lee: None. **A. Bahrami:** None. **M. Vandenberg:** None. **M. McKenna:** None. **H. Zhai:** None. **S. He:** None. **J. Beechem:** None. **P. Divakar:** None. **J. Lyssand:** None. **A. Heck:** None. **K. Young:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.14/D14

Topic: B.10. Neuro-Oncology

Support: SNSF Project Grant

Title: Quantifying information dynamics in controlled neuronal networks during glioblastoma invasion

Authors: *G. AMOS[1], L. JORDI[1], V. VASILIAUSKAITE[2], C. M. TRINGIDES[3];
[1]ETH Zurich, Lab. of Biosensors and Bioelectronics, Zurich, Switzerland; [2]ETH Zurich, Zurich, Switzerland; [3]MSNE, Rice Univ., Houston, TX

Abstract: Glioblastoma (GBM), the most aggressive primary brain tumor in adults, can synaptically integrate into the existing neuronal networks of the brain. This functional connectivity drives tumor growth through increased neuronal excitability, contributing to its poor prognosis with a median survival of only 8 months. These findings suggest that pharmacologically disrupting the functional integration of GBM into surrounding neuronal networks could improve clinical outcomes. However, we still have limited understanding of how GBM impacts local brain networks and studying these complex interactions *in vivo* remains challenging. We present a platform that combines mechanically-tuned hydrogels, multi-compartment microstructures, and long-term electrical monitoring to investigate GBM-neuron interactions *in vitro*. The hydrogels are engineered to match the mechanical properties of tumor microenvironments and are integrated into a polydimethylsiloxane microstructure mounted on a CMOS array, enabling co-culture of human induced pluripotent stem cell-derived neurons with primary GBM cells. The MEAs enable high-resolution tracking of neuronal signaling for two months, generating rich datasets of network dynamics during GBM invasion. By introducing primary GBM cells into mature neuronal networks, we successfully recreate clinically observed electrophysiological patterns, including epilepsy-like activity and enhanced network synchronization. We employ information theory to understand how the primary function of neurons, information transmission, changes during GBM invasion. We find significantly increased active information storage in GBM-infiltrated networks, meaning neurons retain

information longer, and link this to network structure changes using graph theory. Additionally, neurons receive and emit more redundant signals, suggesting GBM reduces information diversity in neuronal networks. This work establishes a 3D *in vitro* model for investigating GBM progression and its effects on neuronal network dynamics, with potential applications in drug development targeting the disruption of tumor-neuron integration. Beyond GBM research, this platform has broad applications for studying how various pathological states modify information processing in neuronal networks.

Disclosures: G. Amos: None. **L. Jordi:** None. **V. Vasiliauskaite:** None. **C.M. Tringides:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.15/D15

Topic: B.10. Neuro-Oncology

Support: NIH R01 NS134410-01
NIH 1R61DC021924-01

Title: Intracranial EEG detection of Connectivity and Spectrotemporal biomarkers that spatially differentiate IDH-mutant and -wildtype glioma infiltration

Authors: *A. C. PAULK[1], S. S. CASH[2], I. DAS[3];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Dept Neurol, Mass Genl Hosp, Boston, MA; [3]Dept. of Neurol., Massachussetts Gen. Hosp., Boston, MA

Abstract: An emerging proposal in brain tumorigenesis is that infiltrating tumor cells alter synaptic connectivity and induce hyperactivity in the neural network, creating a microenvironment that promotes tumor growth. While detailed evidence for this concept is increasing in animal and ex vivo studies, data from human tumors in situ are needed to elucidate the tumor-induced alterations in brain activity that are not easily reproduced ex vivo. To interrogate the underlying microscale changes, we use direct electrical stimulation combined with cutting edge high-resolution microelectrodes to sample brain activity within, adjacent to, and distant from bulk tumor margins. Data from 72 patient participants were analyzed, including both low resolution clinical electrodes (N=40) and microelectrodes with 128-1000 channels printed on ultrathin materials that conform to the cortical surface (N=46), supplemented with Neuropixels microelectrodes recordings (N=29). We compared glioma recordings (N=38; 23 IDH-wildtype [IDHwt], 15 IDH-mutant [IDHm]) to non-tumor control cases (from 28 epilepsy and 6 movement disorder cases). Precise localization of the recording electrodes with respect to brain and tumor structure was achieved through 3D segmentation of pre-operative imaging and mapping of electrode placement in the OR using computer vision techniques. We found that coherence and weighted phase lag index values were significantly different between non-tumor

epilepsy, glioma (including those of different genetic backgrounds), and movement disorder cases. With increasing distance from the tumor edge using measures along the curvature of the cortex, there was a linear decrease in high gamma power in both IDHwt and IDHm gliomas. Similarly, the 1/f exponent of the aperiodic component, commonly representative of the excitatory/inhibitory balance, increased linearly with distance from the tumor boundary ($p<0.005$), suggesting proportionally increased excitation in regions proximal to the tumor. Interestingly, a similar linear relationship existed between Euclidean distance to previous resective or lesion location and this type of activity in non-tumor epilepsy cases ($p>0.005$) but not with movement disorder cases ($p>0.005$). Comparing IDHm to IDHwt tumors, we noted a spatial distribution difference. Changes in high gamma and the 1/f component were more spatially localized in the IDHm cases versus IDHwt. These results begin to provide a high-resolution physiological map of tumor infiltration in the human brain, supporting the proposal that tumor alters neural dynamics while revealing glioma subtype-specific differences in physiology at the tumor boundary.

Disclosures: A.C. Paulk: None. **S.S. Cash:** None. **I. Das:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.16/D16

Topic: B.10. Neuro-Oncology

Support: P01 CA245705

Title: Sex-specific impact of dietary iron levels on Glioblastoma outcomes

Authors: *E. TUFANO[1], K. PALSA[3], R. SERPA[2], T. HELMUTH[4], J. R. CONNOR[3]; [1]Neurosurg., [2]Penn State Col. of Med. Neurosci. Grad. Program, Hershey, PA; [3]Neurosurg., [4]Penn State Col. of Med., Hershey, PA

Abstract: Glioblastoma (GBM) is the most common primary malignant brain tumor in adults with a survival rate ~5%. GBM displays altered iron metabolism essential for tumor growth and proliferation, yet the role of systemic iron on GBM outcomes remains widely debated. Our lab recently explored the association between anemia and tumor outcomes using available TriNetX Research data and saw a sex bias in patient responses. Anemia was associated with reduced median overall-survival (OS) in males but had no significant differences in females. Further, iron supplementation of anemic females trended towards increased median-OS with no change in OS in supplemented males. However, the impact of varying dietary iron levels on tumor growth and survival remains unknown. In this study, we used an in vivo model consisting of C57BL/6 mice and GL261 mouse glioma cells and induced GBM in 12-13-week-old mice by injecting $2*10^4$ GL261 cells intracranially by a stereotaxic apparatus. Mice were placed on control (35 mg/kg iron), iron deficient (3 mg/kg iron) and high iron (350 mg/kg iron) diets after weaning or directly

after tumor injection. Iron deficiency anemia was determined by hemoglobin and hematocrit. Tumor volume was measured 10 days post-injection by magnetic resonance imaging, followed by image segmentation. Iron deficient males showed increased tumor volumes compared to control ($p=0.18$), while iron deficient females had decreased tumor volume compared to control ($p<0.05$). Tumor volumes corresponded to overall survival, with iron deficient males having slightly reduced survival and iron deficient females having slightly increased survival compared to controls. Similarly, males on high iron diet had slightly increased survival while females on high iron diet had no change in survival compared to control. Based on these results, we provide a translational link between clinical observations and show a sex-specific response to dietary iron levels on tumor growth and survival.

Disclosures: E. Tufano: None. **K. Palsa:** None. **R. Serpa:** None. **T. Helmuth:** None. **J.R. Connor:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.17/D17

Topic: B.10. Neuro-Oncology

Title: Histophathological Characterization of Non-Hydrocephalic Symptomatic Pineal Cysts through Digital Pathology

Authors: *T. PETRUCCI[1,2], E. WILSON[3], V. DAYLOR[2], S. DOOLEY[2], C. WELSH[4], S. PATEL[1], R. NORRIS[2,1];
[1]Dept. of Neurosurg., [2]Dept. of Regenerative Med. and Cell Biol., [3]Col. of Med., [4]Pathology and Lab. Med., Med. Univ. of South Carolina, Charleston, SC

Abstract: Non-hydrocephalic symptomatic pineal cysts (nhSPCs) are increasingly recognized in patients presenting with persistent headaches, visual disturbances, and other neurological symptoms; however, the pathological basis of symptom generation remains poorly understood. Current surgical decision-making relies primarily on imaging and clinical presentation, without confirmation of underlying tissue-level abnormalities.
In this study, we performed histopathological analysis of pineal cyst tissue from nhSPC patients ($n=5$) and compared it to control pineal glands ($n=5$) obtained at autopsy. Digital pathology utilizing Pharmanest's FibroNest analysis method revealed that nhSPCs exhibit distinct architectural disruption ($p=0.017$) and significantly altered tissue morphometry ($p=0.047$) compared to controls. Notably, nhSPCs showed significantly increased interstitial fibrosis ($p=0.011$), suggesting a chronic tissue remodeling process.
These findings support the notion that nhSPCs represent biologically active lesions rather than incidental anatomical variants. Our results highlight consistent and clinically relevant structural changes in symptomatic pineal cysts, motivating further mechanistic investigation into nhSPC-associated symptomatology.

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Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.18/D18

Topic: B.10. Neuro-Oncology

Support: NIH Grant NS123385

Title: A fetal oncogene NUAK2 is an emerging therapeutic target in glioblastoma

Authors: *S. M. GLASGOW[1], H. JO[2];
[1]Neurobio., [2]Neurosciences Grad. Program, UCSD, La Jolla, CA

Abstract: Malignant gliomas are highly aggressive primary brain tumors with limited treatment options and poor survival outcomes. Increasing evidence suggests that these tumors hijack neural developmental programs to sustain malignancy. Here, we identify NUAK2, a kinase associated with the HIPPO pathway, as a developmentally regulated gene reactivated in glioma. NUAK2 was robustly expressed in the embryonic mouse brain and fetal human astrocytes but silenced postnatally. Strikingly, NUAK2 expression re-emerges in malignant glioma, correlating with reduced patient survival and reactivation in an adult mouse model of glioma. Functional studies demonstrate that NUAK2 promotes glioma cell proliferation and migration both *in vitro* and *in vivo*. CRISPR-Cas9-mediated deletion of *NUAK2* in glioma cell lines suppressed oncogenic behaviors, while overexpression had the opposite effect. Pharmacological inhibition of NUAK2 also impaired glioma cell proliferation and motility. These findings highlight NUAK2 as a developmentally regulated kinase that is co-opted in glioma and suggest its potential as a therapeutic target in cancer neuroscience.

Disclosures: S.M. Glasgow: None. **H. Jo:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.19/Web Only

Topic: B.10. Neuro-Oncology

Title: Stromal nerve growth factor signaling drives neurovascular remodeling in the gastric cancer tumor microenvironment

Authors: *C. R. DEPINHO[1], S. W. RYEOM[2];
[1]Barnard College, Columbia Univ., New York, NY; [2]Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Gastric cancer (GC) remains a significant global health challenge, with over 1.6 million cases projected annually by 2030. Despite advancements in treatments, survival rates for advanced-stage patients remain persistently low. Poor prognosis is driven in part by complex interactions within the tumor microenvironment (TME), highlighting the critical need for better mechanistic understanding of TME dynamics to develop novel therapeutic strategies. The neurotrophin nerve growth factor (NGF) has been implicated in GC progression, promoting sensory nerve innervation and angiogenesis in GC tumors through its high-affinity receptor, tropomyosin receptor kinase A (TrkA). While NGF has been shown to be expressed in GC tumors, the specific cellular sources of NGF and TrkA in GC remain poorly defined. Leveraging single-cell RNA sequencing data from human GC, we identified mast cells as the predominant cell type expressing TrkA and pericytes as the main source of NGF. These findings gain added significance based on observations in other tumor types where NGF-TrkA signaling in mast cells has been shown to enhance angiogenesis, promote sensory nerve axonogenesis, and modulate immune infiltration, which are hallmark features of GC TME remodeling. Re-analysis of a spatial transcriptomic advanced human GC datasets reveals mast cells are spatially co-localized with neuronal and vascular signatures in the human GC TME, suggesting heterotypic interactions between mast cells and pericytes may drive GC progression, angiogenesis, and axonogenesis through NGF-TrkA signaling. Our results highlight a stromal NGF-TrkA axis that orchestrates neurovascular remodeling and immune dynamics in GC, identifying a clinically actionable target within the tumor microenvironment.

Disclosures: C.R. DePinho: None. **S.W. Ryeom:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR378.20/D19

Topic: B.10. Neuro-Oncology

Title: Electrical Integration of Glioma into Serotonergic Neuronal Circuitry

Authors: *R. DREXLER[1], B. YALCIN[2], R. MANCUSI[3], A. ROGERS[2], K. SHAMARDANI[4], C. LO CASCIO[7], R. C. MALENKA[5], B. D. HEIFETS[2], M. FILBIN[8], K. DEISSEROTH[9], M. MONJE[6];
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[6]Neurol., Stanford Univ., Stanford, CA; [7]Dana-Farber Cancer Inst., Boston, MA; [8]Dana Farber Cancer Inst., Boston, MA; [9]Stanford, Stanford, CA

Abstract: High-grade gliomas are the most aggressive form of brain tumors, and neuronal activity has emerged as a driver of glioma pathophysiology. Activity-dependent glioma growth results from paracrine factor signaling and bona fide neuron-to-glioma synapses that integrate glioma cells into brain-wide neuronal circuits. In turn, gliomas increase neuronal excitability and remodel neural circuits. To date, glutamatergic, GABAergic, and cholinergic neurons have been identified as neuron types that promote glioma proliferation; however, the role of other neuromodulatory circuits remains largely unexplored. Here, we report that glioma cells integrate into serotonergic (5-hydroxytryptamine; 5-HT) midbrain circuits originating from the dorsal raphe nucleus (DR) and median raphe nucleus (MR). Neuronal activity of DR^{5HT} and MR^{5HT}populations drives a robust, calcium-dependent and circuit-dependent increase in the proliferation of diffuse midline glioma and glioblastoma across the neocortex, thalamus, and pons. This proliferative effect is mediated by activation of the $5\text{-}HT_{2A}$ on glioma cell membranes. Knockout and pharmacological blockade of $5\text{-}HT_{2A}$ abrogated the effects of serotonergic neuronal activity and significantly prolonged survival in patient-derived glioma xenograft models. In contrast, the serotonergic psychedelic drug psilocybin persistently enhances glioma cell proliferation and induces sustained calcium transients in a manner dependent on $5\text{-}HT_{2A}$ on glioma cells. As a positive feed-forward mechanism, 5-HT release within the tumor microenvironment increases during glioma progression, contributing to altered activity patterns in distinct 5HT neuron clusters within the DR and MR. This glioma-induced hyperexcitability of 5-HT neurons leads to remodeling of the raphe nuclei and these changes in serotonergic circuit function correlates with the emergence of depressive-like behavior in glioma-bearing mice.Together, these findings uncover the integration of glioma cells into serotonergic circuitry, which contributes to tumor progression and establishes a feed-forward loop between DR^{5HT} and MR^{5HT} long-range projection activity and glioma cells.

Disclosures: R. Drexler: None. **B. Yalcin:** None. **R. Mancusi:** None. **A. Rogers:** None. **K. Shamardani:** None. **C. Lo Cascio:** None. **R.C. Malenka:** None. **B.D. Heifets:** None. **M. Filbin:** None. **K. Deisseroth:** None. **M. Monje:** None.

Poster

PSTR378: Neuro-oncology, Mechanisms, and Biology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.21/D20

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: Support for this study was provided by the Neuroscience program, the College of Medicine, Office of Research and Sponsored Program, the E. Malcolm and Gary Leo Dunbar Endowed Chair, and the John G. Kulhavi Professorship in Neuroscience at CMU.

Title: Co-delivery of kif23 and kif18a siRNAs using g4 70/30 PAMAM dendrimers for glioblastoma treatment

Authors: *N. ALLAHYARZADEH KHIABANI[1,2,3,4], O. DUBEY[1,5], A. POUDEL[2,3,4], B. SRINAGESHWAR[1,2], R. B. PETERSEN[6], G. L. DUNBAR, Sr.[1,7,2,4], J. ROSSIGNOL[2,5,3,4]; [2]Program in Neurosci., [3]Col. of Med., [4]Field Neurosciences Inst. Lab. for Restorative Neurol., [5]Dept. of biochemistry and molecular biology, [1]Central Michigan Univ., Mount Pleasant, MI; [6]Col. of Med., Central Michigan Univ., Mt Pleasant, MI; [7]Dept. of Psychology, Central Michigan Univ., Mount Pleasant, MI

Abstract: Glioblastoma (GB) is the most aggressive and lethal primary brain tumor in adults, characterized by rapid cellular proliferation, pronounced therapeutic resistance, and dismal prognosis. Members of the kinesin superfamily, particularly KIF23 and KIF18A, are markedly overexpressed in GB and play critical roles in mitotic progression and tumor maintenance, making them attractive therapeutic targets. RNA interference (RNAi) using small interfering RNAs (siRNAs) enables gene-specific silencing; however, clinical application is limited by challenges in safe and efficient delivery across the blood-brain barrier (BBB). In this study, we evaluated mixed-surface fourth generation (G4) 70/30 poly(amidoamine) (PAMAM) dendrimers as nanocarriers for the co-delivery of siRNAs targeting Kif23 and Kif18a in both *in vitro* and *in vivo* GB models. Dendrimer-siRNA complexes exhibited optimal physicochemical properties at an N/P ratio of 1.5:1. The complexes demonstrated less cytotoxicity in normal mouse astrocytes while efficiently entering GL261 glioma cells. *In vitro* analyses revealed that co-delivery of Kif23 and Kif18a siRNAs led to gene knockdown, accompanied by upregulation of pro-apoptotic BAX and downregulation of BCL2, suggesting impaired mitotic progression and induction of apoptosis. Wound healing migration assay showed a significant reduction in migratory capacity accompanied by downregulation of MMP2 and MMP9. Notably, expression of stemness markers CD44 and Sox2 remained unchanged, indicating that the dendrimer-siRNA treatment selectively impairs invasive behavior without affecting glioma stemness. *In vivo*, GB mouse model was established via intrastriatal implantation of GL261/luc2-tdtomato cells Tumor engraftment and progression were monitored via IVIS bioluminescence imaging and confirmed by luciferase immunohistochemistry, indicating robust tumor formation. Ongoing studies involve intracranial administration of siRNA-loaded dendrimers to assess their effects on tumor volume, survival outcomes, and molecular markers related to proliferation, apoptosis, invasion, and stemness. These findings underscore the potential of G4 70/30 PAMAM dendrimers as a biocompatible and effective method for co-delivery of siRNA therapeutics. This strategy offers a promising way to suppress key mitotic genes of GB, reduce tumor cell migration, and enhance treatment.

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Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.01/D21

Topic: C.01. Brain Wellness and Aging

Support: NIH 2R15GM123463-02

Title: Exploring the mechanism of action of novel anti-oxidant compounds in the treatment of Alzheimer's Disease

Authors: *G. AKKARAJU;
Texas Christian Univ., Fort Worth, TX

Abstract: Oxidative damage and the subsequent inflammation has beenimplicated in the progression and etiology of Alzheimer's disease. In thisstudy, we explore the mode of action of novel antioxidant compounds (L2 and L3)that have been designed with the dual functionalities of binding metal ions andquenching reactive oxygen species (ROS). One hypothesis is that our compoundsbind to and quench ROS in the cells. Specifically, we examine the effect ofthese compounds in reducing oxidant-induced ROS and ROS-induced cell death inBV-2 mouse microglial cells. The transcription factor Nrf-2 is activated by thepresence of ROS in the cell. Docking studies have shown that our compound canbind to the Nrf-2 regulator KEAP and dissociate Nrf-2 and permit itstranslocation into the nucleus. We examine L2 and L3-induced Nrf-2translocation, using immunofluorescence, and the subsequent downstreamexpression of Nrf-2 activated genes, such as HO-1 and Nrf-2 itself usingqPCR.

Disclosures: G. Akkaraju: None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.02/D22

Topic: C.01. Brain Wellness and Aging

Support: MCPHS University, School of Pharmacy

Title: Cytoprotective effects of Gymnemic Acid 1 in cellular models of neurodegeneration

Authors: *S. KAPSE[1], M. M. MIGLIORE[2];
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Abstract: Gymnema sylvestre (G. sylvestre) is a traditional medicinal herb known for its anti-diabetic properties, yet its molecular mechanisms remain unknown. Growing evidence suggests a strong link between insulin resistance and neurodegeneration, mediated by impaired pro-survival signaling (e.g., PI3K/Akt), increased oxidative stress, and inflammation. This study investigates the cytoprotective potential of Gymnemic Acid 1 (GA1), a key bioactive compound from G. sylvestre, in cellular models of neurodegeneration. Neurotoxicity was induced in SH-SY5Y cells

using either 6-hydroxydopamine (6-OHDA) or streptozotocin (STZ), followed by treatment with increasing concentrations of GA1. GA1 treatment significantly increased cell viability by 25% and reduced malondialdehyde (MDA) levels by 40%, indicating cytoprotective and antioxidant effects. To explore its mechanism of action, we assessed GA1 activity at the glucagon-like peptide-1 receptor (GLP-1R) using a reporter gene assay. GA1 exhibited agonistic activity at GLP-1R, and its protective effects are lost in the presence of a GLP-1R antagonist, further solidifying the involvement of GLP-1R in GA1's action. Further analysis revealed that GA1 treatment increased phosphorylation of PI3K and Akt proteins compared to toxin-only controls, suggesting activation of the GLP-1R/PI3K/Akt signaling pathway. These findings demonstrate that GA1 confers protection against neurotoxic insults via antioxidant mechanisms and activation of GLP-1R-mediated pro-survival signaling. This study provides mechanistic insight into the neuroprotective potential of G. sylvestre and supports further exploration of GA1 as a candidate for therapeutic development in neurodegenerative diseases.

Disclosures: S. Kapse: None. **M.M. Migliore:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.03/D23

Topic: C.01. Brain Wellness and Aging

Support: National Research Foundation of Korea RS-2023-00208884
National Research Foundation of Korea RS-2023-00265824

Title: Comparative Effects of Cognitive Interventions on Cognitive Functions and EEG Spectral Activity in Older Adults: A Crossover Study

Authors: *H. KIM, H. KIM, W. HAN, J. LEE;
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Abstract: With the accelerating pace of population aging, cognitive decline in older adults has emerged as a critical societal concern. Computerized cognitive training (CCT) and non-invasive brain stimulation (NBS) techniques such as transcranial direct current stimulation (tDCS) and transcranial alternating current stimulation (tACS) have gained attention as promising interventions. While these approaches are generally known to enhance cognitive performance, few studies have directly compared their differential effects on specific cognitive subdomains and associated changes in brain activity. This study aimed to investigate the cognitive and electrophysiological effects of CCT, tDCS, and tACS using a crossover design to inform strategies for personalized cognitive interventions. A total of 24 older adults aged 55 and above participated in the study. Each participant received 20-minute sessions of CCT, tDCS, and tACS in a randomized crossover sequence. Pre- and post-intervention assessments included cognitive tasks, such as the N-back test, digit span test (DST), contrasting program, go/no-go test, and

color word Stroop test (CWST), to evaluate specific cognitive functions. Resting-state EEG was recorded for three minutes using a 19-channel EEG system (CGX Quick-20r; Cognionics Inc.), and power spectral density (PSD) was computed for analysis. Results showed improvements across all three interventions in DST and the Contrasting Program. CCT and tACS led to enhancements in the go/no-go test, while CCT and tDCS improved performance on the CWST. EEG analysis revealed increased beta and gamma PSD in the temporal region following CCT, increased delta PSD in the parietal region following tDCS, and increased theta PSD in the lateral parietal region following tACS. These findings suggest that each intervention selectively modulates specific cognitive domains through distinct neurophysiological mechanisms. Moreover, the integration of EEG-based neural markers with behavioral outcomes highlights the potential for developing personalized strategies to optimize cognitive enhancement in aging populations.

Disclosures: H. Kim: None. **H. Kim:** None. **W. Han:** None. **J. Lee:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.04/D24

Topic: C.01. Brain Wellness and Aging

Support: NIH grant

Title: Heart rate variability biofeedback-induced spectral power increase in the baroreflex frequency range predicts orbitofrontal volume changes linked to oddball task performance in older adults

Authors: H. YOO[1], A. J. KIM[1], K. NASHIRO[1], C. CHO[1], N. MERCER[1], M. DAHL[2,1], P. CHOI[1], H. LEE[1], J. THAYER[3], M. MATHER[1];
[1]USC, Los Angeles, CA; [2]Ctr. for Lifespan Psychology, Max Planck Inst. for Human Develop., Berlin, Germany; [3]Univ. of California, Irvine, Irvine, CA

Abstract: When relaxed, breathing makes the heart rate oscillate as heart rate increases during inhaling and decreases during exhaling. Slowing breathing typically increases the amplitude of these oscillations in heart rate (increasing heart rate variability; HRV). Biofeedback that aims to increase the amplitude of heart rate oscillation during slow paced breathing can reduce anxiety, suggesting that it affects anxiety-related brain circuits. Typically, biofeedback involves breathing at about 10 seconds per breath (0.1 Hz), a frequency believed to correspond with the resonance frequency of the baroreflex system. In our 'HRV-ER' clinical trial, we found that biofeedback to increase heart rate oscillation during slow-paced breathing increased left orbitofrontal cortex (OFC) volume compared with biofeedback to decrease heart rate oscillation in both older and younger adults (Yoo et al., 2022).In our subsequent HeartBEAM clinical trial, we examined brain volume changes in adults aged 50-70 undergoing a 10-week HRV biofeedback program

with breathing frequencies customized and adjusted based on performance. Frequency analysis of heart rate data collected during training was used to calculate power within the baroreflex frequency range. Results showed that power achieved in this frequency range during home paced-breathing sessions correlated positively with changes in left orbitofrontal cortex (OFC) volume. Vertex-wise whole-brain analysis revealed significant clusters in medial and lateral left OFC, showing positive correlations between volume changes and power in the upper baroreflex range (0.096-0.13 Hz).To explore frequency-specific effects, we examined correlations between log-transformed power in the upper baroreflex range and volume changes in the two significant OFC clusters. Correlations were strongest at 11-second breathing (~0.09 Hz), a frequency falling within the frequency of the baroreflex. They remained significant at frequencies corresponding with faster breathing (0.1-0.25 Hz) but were not significant at frequencies corresponding with very slow breathing (>16 seconds, <0.06 Hz).Locus coeruleus-noradrenergic system interactions with the prefrontal cortex are related to anxiety. Thus, HRV biofeedback's benefits for anxiety may relate to effects on this circuit. Partial least squares correlation (PLSC) analysis revealed that training-induced OFC volume changes were associated with behavioral and pupillometry measures from an oddball task in addition to the event-related potential P300 component, indicating links between changes in the locus coeruleus system and the observed OFC plasticity.

Disclosures: H. Yoo: None. **A.J. Kim:** None. **K. Nashiro:** None. **C. Cho:** None. **N. Mercer:** None. **M. dahl:** None. **P. Choi:** None. **H. Lee:** None. **J. Thayer:** None. **M. Mather:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.05/D25

Topic: C.01. Brain Wellness and Aging

Support: JSPS KAKENHI/22K07402

Title: Maintaining social networks prevent cognitive decline in oldest old people in Japan

Authors: *H. SHIMAZU[1], M. NAKANO[3], E. KOBAYASHI[2], K. NAGAISHI[2];
[1]Anatomy2, Sapporo Med. Univ., Sapporo, Hokkaido, Japan; [2]Sapporo Med. Univ., Sapporo, Japan; [3]Sapporo Med. Univ. Dept. of Anat., Sapporo City, Hokkaido, Japan

Abstract: Alzheimer's disease (AD) is the primary cause of dementia, believed to be triggered by an accumulation of amyloid-β plaques and neurofibrillary tangles. However, several postmortem brain studies have shown that some cases with AD pathology in the brain maintain normal cognitive function. In this study, we aimed to reveal the psychosocial factors that may prevent the cognitive decline despite the presence of AD pathology. The participants aged 88 and older were recruited from 'Shiragikukai', the body donation organization of Sapporo Medical University. The cognitive function was assessed using the Mini Mental State Examination (MMSE) and the Japanese version of the Montreal Cognitive Assessment (MoCA-J).

Psychosocial functioning was evaluated using Perceived Stress Scale (PSS), Friendship scale, Philadelphia Geriatric Center Morale Scale (PGC), Geriatric depression scale (GDS) and Lubben Social Network Scale (LSNS). In addition, the phosphorylated tau (p-tau) level in saliva samples was evaluated by ELISA. This study was approved by the Sapporo Medical University Ethics Committee (1-2-30), and all of participants consented to the research. The above cognitive and psychosocial functioning has been assessed once a year since 2022, and the second survey had already completed (n = 39). By comparing the results of the first and second year, we divided the participants into two groups, the LSNS maintained group (n = 12) and the LSNS not maintained group (n =27). In the LSNS maintained group, MoCA-J scores in the second year were significantly preserved compared to the LSNS not maintained group. However, there was no significance in the p-tau level in saliva between the two groups in both years. Since the p-tau level in saliva is known to be increased in AD patients, the cognitive function was influenced by not p-tau level but social network. From these results, communications with family and friends may be an important factor to maintain cognitive function, regardless of p-tau level.

Disclosures: H. Shimazu: None. **M. Nakano:** None. **E. Kobayashi:** None. **K. Nagaishi:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.06/D26

Topic: I.04. Executive Functions

Support: IDEA GTO (IDEAGTO/CONV/101/2023)

Title: Cognitive stimulation enhances executive function, well-being, and BDNF levels in older adults: evidence from a quasi-experimental study in a Mexican geriatric center

Authors: *N. Y. CORTÉS[1], C. R. VUELVAS-OLMOS[2];
[1]Univ. de Guanajuato, Guanajuato, Mexico; [2]Univ. of Colima, Colima, Mexico

Abstract: The progressive increase in the aging population highlights the need for interventions aimed at preserving cognitive health and overall wellbeing in older adults. This study aimed to assess the impact of a structured cognitive training program on psychological well-being, executive function performance, and Brain Derived Neurotrophic Factor levels (BDNF) in older adults from a Mexican geriatric center. A quasi-experimental pretest-posttest design with a nonequivalent control group was conducted. Thirty-two older adults were assigned either to a cognitive stimulation intervention group or a control group. The intervention consisted of 120 individually structured sessions, each lasting approximately 60 min, delivered five times per week over 24 weeks. Independent neuropsychologists, blinded to group allocation, assessed executive function (BANFE-3), depressive symptoms (Yesavage Geriatric Depression Scale), autonomy in daily living (Barthel Index), and quality of life (WHOQOL-OLD) before and after the intervention. Serum BDNF levels were also measured. The intervention group showed

significant improvements in executive function, depressive symptoms, independence in daily activities, and quality of life, while the control group showed no changes. Additionally, the intervention group showed an increase in BDNF expression post-intervention. The cognitive stimulation program effectively improved cognitive performance, emotional well-being, autonomy, and quality of life in older adults. These results support the development and implementation of cognitive stimulation strategies as viable and effective tools for preventing cognitive decline and promoting general well-being in old age. They also open up new lines of research on how behavioral interventions can influence neurobiological biomarkers such as BDNF, which is of great interest for the design of therapeutic programs in the field of clinical and translational neuroscience.

Disclosures: N.Y. Cortés: None. **C.R. Vuelvas-Olmos:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.07/D27

Topic: C.01. Brain Wellness and Aging

Support: Lewis College Foundation Award

Title: The impact of Juçara berry supplementation on memory in male mice consuming a western diet

Authors: *S. M. VARGHESE[1,2], W. ELAMAD[1,3], J. P. DANH[1,3], A. P. S. SIQUEIRA[3,4], A. M. MABB[2], D. WANDERS[1,2,3], R. G. FERESIN[1,2,3];
[1]Chem., [2]Neurosci. Inst., [3]Nutr., Georgia State Univ., Atlanta, GA; [4]Inst. Federal Goiano, Atlanta, Brazil

Abstract: Objectives Westernized diet (WD), high in fat, salt and sugar, is linked to cognitive decline as it induces inflammation and oxidative stress in the hippocampus, a vital learning and memory site. Juçara berry (JU) (*Euterpe edulis*), native to Brazil, contains polyphenols with antioxidative, anti-inflammatory, and neuroprotective properties. Thus, the objective of this study was to assess the effects of JU supplementation on memory in male mice consuming WD. **Methods** Four-week-old male UM-HET3 mice, genetically heterogenous strain, were fed an AIN-93M (low-fat, low-sucrose, low-salt; LFLSS) diet alone or supplemented with 5% (w/w) JU (LFLSS + JU) for 4 weeks. At week 5, mice on LFLSS diet continued on the diet or switched to high-fat (40%), high-sucrose (45%), high-salt (8%) (HFHSS) diet. Mice consuming LFLSS + JU switched to HFHSS diet supplemented with 5% (w/w) JU (HFHSS + JU). The novel object (NO) recognition (NOR) test was performed on week 9. On day 1 of NOR, mice explored a testing box for 15 min. On day 2, mice explored two identical familiar objects (FO) in the testing box for 15 min. After 5 h, one of the FO was replaced with a NO. Mice were sacrificed on week 16, hippocampi were extracted and protein expression assessed using western blot. **Results** No

significant differences were observed in time spent investigating the NO among groups. HFHSS showed no significant difference in time spent investigating the FO compared to LFLSS (p=0.99, d=0.05) and HFHSS + JU (p=0.5, d=0.5). HFHSS + JU had no distinguishable trend in preference for NO compared to LFLSS (p=0.4, d=0.6) and HFHSS (p=0.8, 0.3). Latency to investigate the NO was significantly lower in LFLSS compared to HFHSS (p=0.03, d=1.5) but was not significantly different compared to HFHSS + JU (p=0.2, d=0.9). There was not a distinguishable trend in the latency to investigate the FO in LFLSS compared to HFHSS (p=0.2, d=0.9). Expression of glial fibrillary acid protein, neuronal nuclear protein, and brain-derived neurotrophic factor were not affected.
Conclusion Our data indicate that JU consumption does not significantly affect curiosity, memory, and cognitive processing in mice consuming WD; however, most effect sizes observed were moderate to large and will inform sample size calculations for future studies aiming to examine effects of similar functional food interventions on behavior, and inflammatory pathways.

Disclosures: S.M. Varghese: None. **W. Elamad:** None. **J.P. Danh:** None. **A.P.S. Siqueira:** None. **A.M. Mabb:** None. **D. Wanders:** None. **R.G. Feresin:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.08/Web Only

Topic: C.01. Brain Wellness and Aging

Support: Fresh Pond Research Institute

Title: Taurine modulates TRH and TRH-like peptide expression throughout the brain and peripheral tissues of male rats

Authors: *A. E. PEKARY;
VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

Abstract: Serum levels of taurine, a semi-essential amino acid, declines by 80% in mice, monkeys and humans with aging. Taurine has therapeutic effects ranging from treatment of depression, Alzheimer's and Parkinson's disease, obesity, diabetes, neurodegeneration and aging. TRH and TRH-like peptides, with the structure pGlu-X-Pro-NH_2, where "X" can be any amino acid reside, have reproductive, caloric-restriction-like, anti-aging, pancreatic-β cell-enhancing, cardiovascular, and neuroprotective effects. The TRH/TRH-R1 receptor signaling pathway is an important mediator of brain-gul axis communication via the medulla oblongata. TRH and TRH-like peptides occur not only throughout the CNS but also peripheral tissues, with particularly high levels in rat and human prostate. Sixteen young adult male Sprague-Dawley rats were divided into 4 groups. Control group remained on ad libitum chow and water for 10 days. Acute group received ad libitum chow and water for 9 days and then 2.5% taurine in rat chow for

24 h. Chronic animals received taurine in chow for 10 days. Withdrawal rats received taurine chow for 8 days and then normal chow for 2 days. TRH and TRH-like peptide levels were measured in medulla oblongata (MED), frontal cortex (FCX), hypothalamus (HY), amygdala (AY), hippocampus (HC), piriform cortex (PIR), nucleus accumbens (NA), entorhinal cortex (ENT), striatum (STR), cerebellum (CBL), anterior cingulate (ACNG), posterior cingulate (PCNG), prostate (PR), liver (L), testis (T), heart (H), pancreas (PAN), adrenals (AD) and epididymis (EP) by combined HPLC and RIA. Significant changes in the levels of TRH and TRH-like peptides occurred throughout the brain and peripheral tissues in response to taurine treatment by one way ANOVA using Scheffe post hoc comparison with the corresponding control group. The number of significant changes in TRH and TRH-like peptide levels in brain resulting from taurine treatment, in descending order were: CBL (8), FCX (6), AY (5), HC (4), PCNG (3), MED (1), ACNG (1), HY (0), PIR (0), ENT (0), STR (0). The corresponding ranking for peripheral tissues was: L (7), EP (6), PAN (4), PR (1), AD (1), H (1), T (0). Conclusion: The sensitivity of TRH and TRH-like peptide expression to taurine treatment, particularly in the CBL, FCX, AY and HC in brain and L, EP and PAN in peripheral tissues is consistent with TRH and TRH-like peptides participating in the therapeutic effects of taurine on neurological and metabolic disorders, and male reproduction.

Disclosures: A.E. Pekary: None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.09/D28

Topic: C.01. Brain Wellness and Aging

Support: FAPESP 2022/09277-4
FAPESP: 2018/07366

Title: Prolonged Caffeine Intake Modulates synaptic Function and Plasticity in Aging

Authors: *G. S. VILAR HIGA[1], R. DE PASQUALE[2];
[1]Biochem., Univ. de São Paulo, São Paulo, Brazil; [2]Physiol. and Biophisics, Biomed. Sci., Universidade de São Paulo, Brazil

Abstract: The aging process in the brain initiates a cascade of changes that predispose it to pathological alterations, notably memory deficits. Explicit memory formation is closely linked to the neural functions of the hippocampal formation, where synaptic plasticity serves as the primary mechanism for reorganizing circuitry to facilitate the consolidation and retrieval of information acquired through experience. During aging, changes in hippocampal circuit excitability and neuronal communication contribute to the impairment of mnemonic processes. Adenosine (ADO) neuromodulation is critical in this context, as it exerts a modulatory effect by regulating neurotransmitter release. Furthermore, the adenosinergic system undergoes significant

alterations as the brain ages. We hypothesized that targeting the adenosinergic system could enhance neuronal activity and synaptic plasticity within the hippocampus. To test this hypothesis, we employed the senescence-accelerated mouse model prone 8 (SAMP-8) to investigate the prolonged effects of chronic caffeine consumption—a non-specific antagonist of ADO receptors and a widely consumed compound. We assessed the intrinsic excitability and synaptic mechanisms of CA1 pyramidal neurons using intracellular patch-clamp whole-cell recordings. Our findings reveal that caffeine consumption increases neuronal firing activity while downregulating presynaptic glutamate release compared to untreated aged control animals. Additionally, caffeine treatment heightens endogenous A1 receptor-mediated inhibition in neurons from untreated animals. Notably, we were unable to induce long-term potentiation (LTP) in control animals under 50 Hz stimulation of the Schaffer collateral, a deficit that was reversed in the caffeine-treated group. Together, these results suggest that chronic inhibition of ADO receptors may enhance hippocampal mnemonic processes by increasing neuronal excitability, reducing basal neuronal synaptic activity, and facilitating LTP in aged subjects. This work was funded by FAPESP (process n° 2022/09277-4; 2018/07366-42023/02046-0)

Disclosures: G.S. Vilar Higa: 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.; FAPESP 2022/09277-4, CNPQ, CAPES. **R. De pasquale:** A. Employment/Salary (full or part-time): Universidade de São Paulo. 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.; FAPESP, CNPQ, CAPES.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.10/D29

Topic: C.01. Brain Wellness and Aging

Support: NIH R01AG071787

Title: Probiotic supplementation restores microglial complexity in aged mice

Authors: *H. LEE, Y. ZUO;
Univ. of California Santa Cruz, Santa Cruz, CA

Abstract: Probiotics are live microorganisms that provide health benefits to the host, in part by modulating the gut microbiota. Recent studies have highlighted the role of the gut-brain axis in regulating central nervous system immune responses through microbial modulation. Aging is associated with morphological and functional changes in microglia, the resident immune cells of the brain, which can contribute to neuroinflammation and cognitive decline. However, little is

known about how probiotics directly affect microglial morphology in the aging brain. To address this, we utilized in vivo two-photon microscopy with a thin-skull preparation to monitor microglial dynamics in aged mice following probiotic treatment. Aged mice were administered a probiotic cocktail for three weeks. Morphological analysis revealed a significant increase in microglial branch number, total process length, number of junctions, and endpoints compared to control aged mice. Sholl analysis demonstrated a significant increase in the number of total intersections, indicating enhanced microglial complexity. Furthermore, we analyzed the dynamics of microglial tips over a 25-minute period at 5-minute intervals. While the rate of change did not differ between aged mice treated with probiotics and untreated controls, the probiotic-treated group exhibited a greater net extension of microglial tips by the end of the observation period. Taken together, our findings suggest that probiotic supplementation increases the structural complexity of microglia in aged mice, restoring their morphology to a state that closely resembles that of younger animals. Further studies are needed to elucidate the underlying mechanisms by which probiotics modulate microglial structure and function in the aging brain.

Disclosures: H. Lee: None. **Y. Zuo:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.11/D30

Topic: C.01. Brain Wellness and Aging

Support: Brains & Behavior Seed Grant

Title: Impact of Tocotrienol Supplementation on Neuroinflammation and Central Insulin Resistance in Neuronal Cells and Mouse Brain

Authors: *E. SHAH[1], R. LAWRENCE[1], F. PINHEIRO[5], H. L. LAIL[2], A. M. MABB[3], W. XIA[6], J. CAO[7], D. WANDERS[4];
[2]Chem. | Nutr., [3]Neurosci. Inst., [4]Nutr., [1]Georgia State Univ., Atlanta, GA; [5]Univ. of Porto, Porto, Portugal; [6]GRECC, Pharmacol. & Exptl. Therapeut., Sch. of Med., Bedford, MA; [7]USDA, North Dakota, ND

Abstract: Obesity and type 2 diabetes contribute to cognitive dysfunction through multiple mechanisms including neuroinflammation and central insulin resistance. Tocotrienols (T3), a form of vitamin E, have demonstrated beneficial effects on peripheral insulin sensitivity and inflammation, but their impact on central insulin signaling remains underexplored. Neuro2a (N2a) cells were pretreated with either vehicle or δ-T3 (10 μM). Cells were then exposed to either control media or conditioned media (CM) derived from LPS-activated RAW 264.7 macrophages, with the continued presence of δ-T3 or vehicle. Prior to lysate collection, N2a cells were treated with either vehicle or insulin (100 nM). Western blotting was used to assess total and phosphorylated forms of proteins involved in the insulin signaling cascade. Gene expression

related to inflammation and insulin/glucose metabolism was measured using real-time PCR. To test this pathway in vivo, male C57BL/6J mice were assigned to one of three dietary groups: 1) low-fat diet (LFD), 2) high-fat diet (HFD), or 3) HFD supplemented with tocotrienols (800 mg/kg diet). Body weight and adiposity were assessed weekly. Inflammatory and insulin signaling markers in the brain were measured via PCR, multiplex protein assay, and western blotting. Proteomic analysis of brain tissue was also conducted. δ-T3 increased basal Akt phosphorylation and mitigated inflammation-induced insulin resistance in N2a cells. δ-T3 also significantly attenuated CM-induced increases in *Il6, Il23,* and *Nfkb*. HFD intake significantly increased body weight and adiposity. T3 supplementation prevented HFD-induced reductions in hypothalamic *Irs2* expression, suggesting that T3 promotes healthy brain insulin signaling. Proteomic analysis found that HFD consumption altered pathways associated with lipid transport and metabolism, while tocotrienol consumption altered ion binding pathways in the brain. Altogether, our findings suggest that δ-tocotrienol protects from inflammation-induced central insulin resistance *in vitro* and *in vivo*, highlighting its potential neuroprotective role in metabolic disorders.

Disclosures: E. Shah: None. **R. Lawrence:** None. **F. Pinheiro:** None. **H.L. Lail:** None. **A.M. Mabb:** None. **W. Xia:** None. **J. Cao:** None. **D. Wanders:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.12/D31

Topic: C.01. Brain Wellness and Aging

Support: NIH MH106640,
AA 23AARGD-NTF-1027173
VA BRAVE

Title: Long-term carbamoylated erythropoietin administration preserves cognitive function in aged mice

Authors: *J. SADANANDAN, M. SATHYANESAN, S. S. NEWTON;
Div. of Biomed. & Translational Sci., The Univ. of South Dakota, Vermillion, SD

Abstract: The aging of the brain is a multifactorial process, often accompanied by progressive cognitive decline and an increased susceptibility to neurodegenerative disorders. Neurotrophic factors play a crucial role in supporting neuronal health and preserving cognitive abilities throughout the lifespan. Among these, erythropoietin (EPO) has emerged as a key player due to its neuroprotective and neurotrophic properties. However, age-related reductions in EPO expression, particularly in the hippocampus, have been implicated in cognitive decline. Although EPO administration has demonstrated cognitive benefits in preclinical models, its clinical application is limited due to strong hematopoietic effects. To address this limitation, we

investigated carbamoylated erythropoietin (CEPO), a non-hematopoietic derivative of EPO that retains neurotrophic activity, as a potential therapeutic candidate for age-associated cognitive decline. We used C 57 mice for our studies and grouped them into a control group, administered with PBS, and a long- term CEPO administration group starting from 12 months of age, followed by assessments of cognitive functions at 16,,18, and 20 months using various behavioral tests, including open- field, novel object recognition (NOR), novel object location (NOL), and the Morris water maze (MWM). At 16 months, no significant differences were observed between the groups. However, from 18 months onward, CEPO-treated mice exhibited improved object recognition, indicating early cognitive preservation. By 20 months, CEPO-treated mice demonstrated significantly enhanced spatial learning and memory, as evidenced by superior MWM performance. Fluorescent in situ hybridization revealed EPOR expression predominantly in the dentate gyrus, and CD 131 localized in the hilus, suggesting that CEPO primarily signals through EPOR homodimers rather than EPOR-CD 131 heterodimers. These findings demonstrate that CEPO attenuates age-related cognitive decline in mice, likely via hippocampal EPOR-mediated signaling, and support its potential as a therapeutic candidate for age-related cognitive disorders.

Disclosures: J. Sadanandan: None. **M. Sathyanesan:** None. **S.S. Newton:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.13/D32

Topic: C.01. Brain Wellness and Aging

Title: Does metformin influence the risk of postoperative delirium and mortality? Insights from a decade of surgical data

Authors: *N. KAJITANI, T. YAMANASHI, D. YOSHIOKA, K. KOMATSU, C. USHIDA, M. NAKAMOTO, M. IWATA;
Tottori Univ., Yonago, Japan

Abstract: Objective: Although our previous study suggested that metformin might be associated with a reduced incidence of delirium and reduced mortality (Yamanashi et al., Aging, 2022), there were several limitations including unclear information about metformin use at the time of the survey, failure to account for the use of diabetes medications other than metformin and insulin, and the omission of other medications that could influence the risk of delirium. To overcome these limitations, we conducted a detailed analysis of the medical records of surgical patients to evaluate the effect of metformin on the occurrence and prognosis of postoperative delirium. Method: This retrospective cohort study included patients aged 65 years or older with type 2 diabetes mellitus (T2DM) who underwent surgery for malignant tumors at the Department of Digestive Surgery or Thoracic Surgery at Tottori University Hospital between January 2012 and December 2022. A total of 1,159 patients were analyzed. Medical records were reviewed to

extract data on age, sex, BMI, comorbidities, medications, blood test results, surgical procedure, blood loss, anesthesia time, and other factors. The relationships between metformin use and the risk of delirium, as well as between metformin use and mortality, were examined. Results: Of the 1,159 patients, 1,026 were classified into the non-metformin group, and 133 were classified into the metformin group. The prevalence of delirium was 17.0% in the non-metformin group and 15.0% in the metformin group. After adjusting for confounding factors, a history of metformin use did not significantly reduce the risk of delirium. Similarly, the 3-year mortality rate did not differ significantly between the metformin and non-metformin groups. Use of metformin use showed no association with reduced mortality after adjusting for confounding factors. Conclusion: Our findings suggest that metformin use does not influence the risk of postoperative delirium or mortality in patients with T2DM. It might be possible that the effects of metformin vary depending on clinical settings and patient characteristics. This study was approved by the Ethics Committee of the Tottori University Faculty of Medicine.

Disclosures: N. Kajitani: None. **T. Yamanashi:** None. **D. Yoshioka:** None. **K. Komatsu:** None. **C. Ushida:** None. **M. Nakamoto:** None. **M. Iwata:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.14/D34

Topic: C.01. Brain Wellness and Aging

Support: Research funding from Lotte Co. Ltd (to H. H. and Y. O.)

Title: Effects of mastication training on resting-state functional connectivity and cognitive preservation in older adults

Authors: *Y. HAYASHI[1], M. SHIROBE[2], M. IWASAKI[2,3], K. MOTOKAWA[2], T. GOMI[2], A. EDAHIRO[2], J. KAWAMURA[4], S. KANNO[4], K. OKABAYASHI[4], H. HIRANO[2], Y. ONO[1]; [1]Meiji Univ., Kawasaki, Japan; [2]Tokyo Metropolitan Inst. for Geriatrics and Gerontology, Itabashi, Japan; [3]Hokkaido Univ., Sapporo, Japan; [4]LOTTE CO., LTD. Res. and Develop. Ctr., Saitama, Japan

Abstract: Aging is associated with declines in higher-order cognitive brain networks, including the Default Mode Network (DMN), Salience Network (SN), and Central Executive Network (CEN). This study aimed to investigate the effects of mastication training on these networks in older adults using a single-arm pre-post design. Ten participants (age range 68-89 years; mean=79.2, standard deviation=8.6; 6 females) completed a one-month mastication training, chewing two pieces of lemon-flavored gum three times daily after each meal. Cognitive performance was assessed using the Trail Making Test (TMT-A/B), and resting-state functional magnetic resonance imaging was conducted at baseline and after the intervention to evaluate changes in brain network activity. Changes in average functional connectivity (FC) within and

between the three networks were assessed using the Wilcoxon signed-rank test. A significant increase in intra-network FC within the SN ($p < 0.05$, uncorrected) was observed only in participants who showed improved TMT-A performance after the intervention (N=7). The post-intervention improvement in TMT-A performance was further correlated with the increased FC between the right anterior insula and the left dorsolateral prefrontal cortex, which are core hubs of the SN and CEN, respectively (cluster-level FDR $p < 0.05$, peak Z-score = 4.24, cluster size = 75). These findings suggest that the effects of the mastication training were most pronounced within the SN, and that cognitive gains may have been mediated by enhanced FC both within the SN and between the SN and executive control regions such as the left dorsolateral prefrontal cortex. These results provide novel insights into the neural mechanisms underlying mastication-induced enhancement in complex information processing. Targeted behavioral interventions may offer a novel approach to maintaining brain wellness in older adults by modulating brain networks involved in higher-order cognition.

Disclosures: Y. Hayashi: None. **M. Shirobe:** None. **M. Iwasaki:** None. **K. Motokawa:** None. **T. Gomi:** None. **A. Edahiro:** None. **J. Kawamura:** A. Employment/Salary (full or part-time): LOTTE CO., LTD. **S. Kanno:** A. Employment/Salary (full or part-time): LOTTE CO., LTD. **K. Okabayashi:** A. Employment/Salary (full or part-time): LOTTE CO., LTD.. **H. Hirano:** None. **Y. Ono:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.15/D35

Topic: C.01. Brain Wellness and Aging

Title: Effects of Exercise Preconditioning on Promoting Endogenous Recovery Responses After Ischemic Stroke in Middle-Aged Mice

Authors: *N. FUKUDA[1,2], H. TAKASE[2], H. YOSHIOKA[1], H. KINOUCHI[1], E. H. LO[2], K. ARAI[2];
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Abstract: Background: Ischemic stroke, caused by the occlusion/stenosis of cerebral blood vessels, results in significant neurological damage. Evidence suggests that post-stroke rehabilitation with physical activity mitigates the effects of ischemic stroke. However, post-stroke neurological deficits make exercise challenging in the clinical setting. Despite increasing attention to the potential benefits of pre-stroke exercise, even in preclinical studies, its effects in middle-aged mice remain poorly understood. **Aim:** This study aimed to investigate the effects of treadmill exercise preconditioning on ischemic brain injury in middle-aged mice using the MCAO model. **Methods:** Early stages of middle-aged (8-month-old) male mice were assigned to

sedentary or exercise groups. After 5 weeks of treadmill running (5 days per week after 1 week of acclimatization), mice underwent 45 minutes of MCAO and reperfusion. Behavioral assessments and body weight monitoring were performed before and after MCAO. Brain analyses for infarct volume and cellular responses were performed 14 days after stroke. **Results:** Fourteen days after stroke, lesion volume was significantly reduced in the exercise group compared to sedentary controls. Exercise preconditioning enhanced neurogenesis, angiogenesis, and oligodendrogenesis while suppressing reactive astrocyte activation. Improved neurological outcomes were also observed in the exercise group. **Conclusions:** Preconditioning with treadmill exercise provides neuroprotection against ischemic stroke by reducing infarct volume and promoting recovery. These findings suggest that daily exercise may be effective in reducing ischemic brain damage, even in middle-aged populations.

Disclosures: N. Fukuda: None. **H. Takase:** None. **H. Yoshioka:** None. **H. Kinouchi:** None. **E.H. Lo:** None. **K. Arai:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.16/D36

Topic: C.01. Brain Wellness and Aging

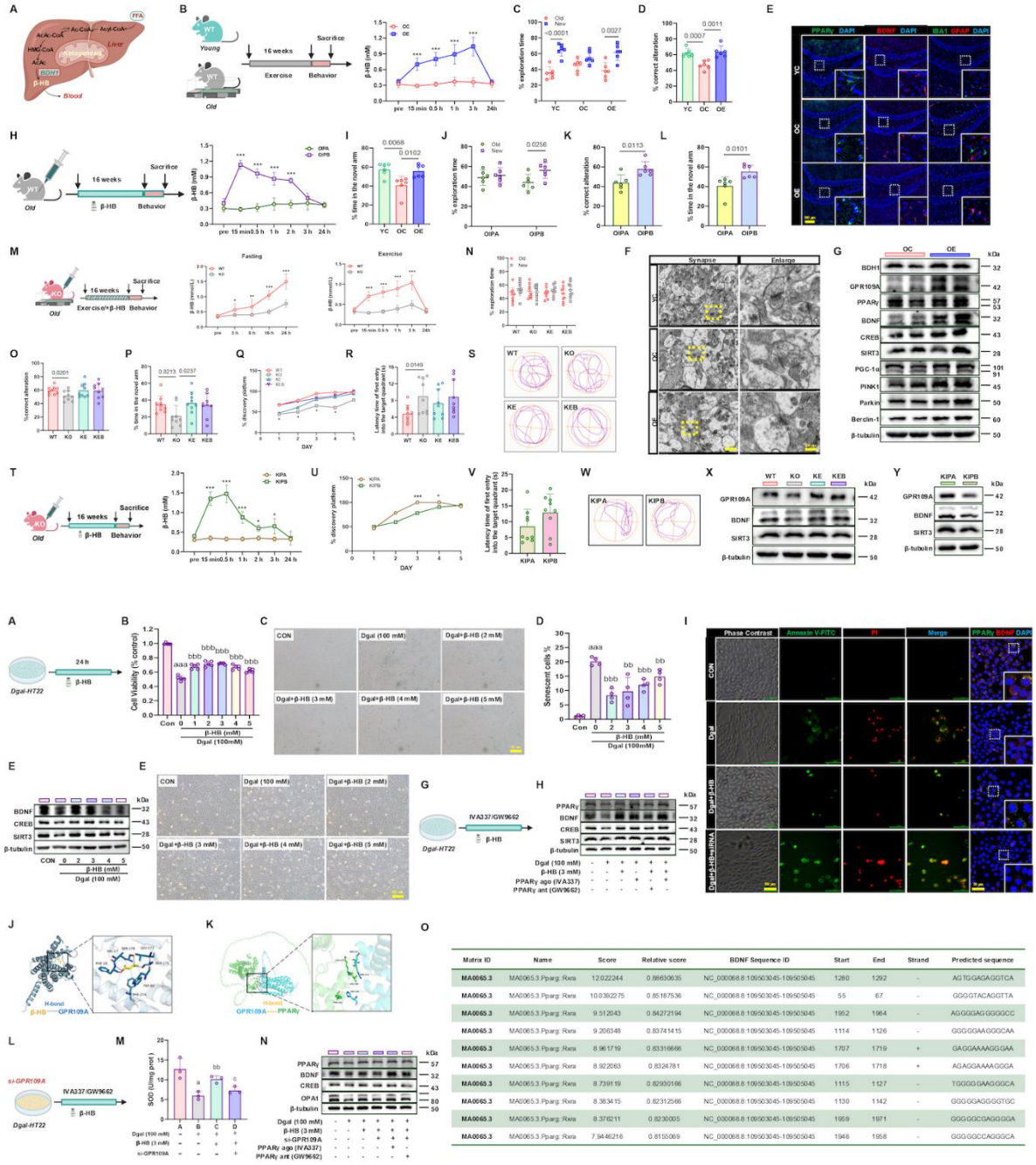
Support: National Natural Science Foundation of China 32371185

Title: Aerobic exercise exerts neuroprotective effects in aging mice via the β-HB/GPR109A-PPARγ signaling axis

Authors: *L. WANG[1], L. MAO[1], Q.-G. ZHANG[2], K. M. DHANDAPANI[1], W. XIAO[3]; [1]Dept. of Neurol., Augusta Univ., Augusta, GA; [2]Dept. of Neurol., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA; [3]Sch. of Exercise and Hlth., Shanghai Univ. of Sport, Shanghai, China

Abstract: Aging is a major contributor to cognitive decline, yet effective strategies remain limited. We investigated whether β-hydroxybutyrate (β-HB), a ketone body elevated by exercise, promotes neuroprotection in aging via the GPR109A-PPARγ signaling axis. In aged wild-type mice, aerobic exercise increased endogenous β-HB levels and enhanced recognition memory, working memory, and hippocampal plasticity, along with upregulation of PPARγ, BDNF, and mitochondrial regulators. Exogenous β-HB supplementation mimicked these effects. However, in BDH1-knockout mice, the loss of β-hydroxybutyrate dehydrogenase 1 (BDH1)—a key enzyme in β-HB synthesis—impaired β-HB production and reduced the neuroprotective effects of both exercise and β-HB supplementation. In vitro, β-HB protected D-galactose-induced senescent HT22 cells by activating antioxidant and mitochondrial pathways through PPARγ. GPR109A knockdown impaired β-HB-induced PPARγ activation, reducing neurotrophic support and increasing apoptosis and inflammation. These results identify β-HB as a key mediator of exercise-induced neuroprotection, with GPR109A-PPARγ signaling essential for its beneficial

effects, and highlight β-HB as a potential intervention against brain aging and cognitive impairment.



Disclosures: L. Wang: None. **L. Mao:** None. **Q. Zhang:** None. **K.M. Dhandapani:** None. **W. xiao:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.17/D37

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant DA049260
NIH Grant DA038092

Title: Ketogenic Diet impact on Long-Term Potentiation in the Dorsal CA1 Hippocampal Region and Memory Behavior in Young and Adult Rodents

Authors: *A. TERRY[1], J. WEIGHT[1], M. DEW[5], E. SAITO[2], B. REED[3], A. C. EVERETT[4], J. G. EDWARDS[2];
[2]Cell Biol. and Physiol., [3]Neurosci. Ctr., [1]Brigham Young Univ., Provo, UT; [4]Brigham Young Univ., Lehi, UT; [5]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: The ketogenic diet (KD), initially developed as a treatment for epilepsy, has recently gained popularity for its potential in promoting weight loss. However, its broader effects on neurological function remain unclear. In this study, we investigated how the KD influences cognition and synaptic plasticity by assessing long-term potentiation (LTP) in the CA1 region of the hippocampus in both young (2-8 weeks) and adult (6-7 months) C57BL/6 mice. Two approaches were used to elevate ketone levels: (1) a high-fat diet administered for 3-4 weeks to induce systemic ketosis, and (2) ex vivo exposure of hippocampal slices to beta-hydroxybutyrate (BHB)-enriched artificial cerebrospinal fluid (ACSF), achieving higher ketone concentrations than are possible through diet alone. Dietary ketosis in rodents only raises blood ketone levels to about 2mM which is lower than levels commonly observed in humans. To ensure unbiased data collection, all experiments were conducted under blinded conditions using field electrophysiological techniques. In young mice, the KD did not produce a significant effect on hippocampal LTP compared to controls. However, adult mice given the KD showed significant enhancement in LTP (181 ± 18%; $n=10$; $p<0.05$) compared to the mice fed a control diet. Similarly, hippocampal slices from young mice exposed to 7.5mM BHB and 2.5mM glucose for over 2 hours showed no significant difference in LTP compared to control slices (0mM BHB, 11mM glucose). There were also no notable sex-based differences in LTP among mice treated with either BHB or control ACSF. Experiments determining whether BHB exposure affects LTP in hippocampal slices from adult mice and whether there are sex-specific effects in this age group are ongoing and statistical analysis is underway. We also conducted behavioral testing using the Morris water maze to assess spatial memory in both age groups following 3-4 weeks of KD administration. No significant differences ($p>0.05$) were observed in the time to locate the submerged platform or time spent in the target quadrant when comparing KD-fed and control mice in the younger cohort ($n=11$ KD, $n=12$ control). In a previous publication, we identified age-related differences in memory performance using the novel object recognition test between adult and young mice (Sait et al., 2022, Metabolites). Taken together, our results suggest that the KD may enhance certain aspects of cognitive and synaptic function in adult mice, while having limited impact on younger animals.

Disclosures: A. Terry: None. **J. Weight:** None. **M. Dew:** None. **E. Saito:** None. **B. Reed:** None. **A.C. Everett:** None. **J.G. Edwards:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.18/D38

Topic: C.01. Brain Wellness and Aging

Support: NIH R01AG076704
NIH T32HL007901

Title: Examining the Effects of the Ketogenic Diet on Burst Suppression and Cognitive Recovery Following Anesthesia in Aged Rats

Authors: *A. R. LARK[1], F. A. MIR[1], F. TIAN[1], N. AMBAREEN[2], J. CHOI[1], A. M. CARPENTER[3], K. SMITH[3], E. CROWLEY[3], B. TORO[3], C. SYME[3], C. J. NEHS[4,1]; [1]Massachusetts Gen. Hosp., Boston, MA; [2]CSIR-Indian Inst. of Chem. Biol., Kolkata, ; [3]Brigham Young Univ., Provo, UT; [4]Harvard Med. Sch., Cambridge, MA

Abstract: Metabolism is increasingly recognized as a critical factor in aging and dementia. The brain's high energy demands make it particularly vulnerable to age-related metabolic alterations that destabilize its energy supply. Notably, glucose metabolism declines with age, limiting the brain's primary energy source. However, the brain retains the ability to utilize ketone bodies, which are generated predominantly by the hepatic β-oxidation of fatty acids, with minor contributions from astrocytic ketogenesis. Nutritional ketosis, induced by a ketogenic diet, enhances ketone availability and has been shown to delay the onset of general anesthesia. Elevated burst suppression during anesthesia is associated with postoperative delirium and is hypothesized to reflect cerebral energy deficits. We therefore hypothesized that a ketogenic diet could provide additional energy resources and in turn reduce levels of burst suppression during anesthesia. We therefore investigated how dietary ketosis alters brain oscillations during and after anesthesia. Young adult Fischer 344 rats were fed either a ketogenic diet (n=9) or control diet (n=8) for ≥60 days. Electroencephalogram (EEG) and electromyogram (EMG) recordings were conducted during two hours of 3% sevoflurane anesthesia. Ketogenic diet-fed rats exhibited a significant reduction in burst suppression, suggesting improved cerebral metabolic stability (two sample t-test, p=0.007). To extend these findings to aged animals, we studied male and female Fischer 344 rats aged ≥22 months. Rats were fed a ketogenic (females n=9, males n=6) or control diet (females n=8, males n=6) for ≥60 days. EEG/EMG electrodes were implanted, and burst suppression latency, rate, and duration were assessed during 2 hours of 3% sevoflurane anesthesia, both before and after dietary intervention. We also recorded EEG oscillations for over 6 hours post-anesthesia to examine acute recovery oscillation dynamics. Behavioral assessments, including the open field test, novel object recognition, and Y-maze, were conducted ~24 hours before, and at 6 and 24 hours after anesthesia to evaluate cognitive outcomes. Our

findings revealed that unlike in young animals, a ketogenic diet intervention did not significantly improve burst suppression measures. However, modest improvements were observed in select cognitive behavioral measures. These findings suggest that while ketones may support some aspects of early post-anesthetic cognitive recovery in aging brains, these benefits are not necessarily mirrored in neural oscillatory activity during anesthesia. Moreover, extreme old age may attenuate the efficacy of ketogenic interventions.

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Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

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Program #/Poster #: PSTR379.19/D39

Topic: C.01. Brain Wellness and Aging

Support: National Institute on Aging Grant R01AG075440

Title: Extracellular Vesicles from Human iPSC-Derived Neural Stem Cells Can Slow Down the Progression of Brain Aging

Authors: *S. ATTALURI, G. SHANKAR, M. KODALI, L. N. MADHU, V. RAO, S. RAO, B. SHUAI, Y. SOMAYAJI, P. PANDA, A. K. SHETTY;
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Abstract: Brain aging, the leading risk factor for developing neurodegenerative diseases, is characterized by progressive cognitive decline associated with moderate neuroinflammation, enhanced mechanistic target of rapamycin (mTOR) signaling, reduced autophagy, and diminished hippocampal neurogenesis. Approaches that effectively mitigate the progression of such age-related changes can improve the quality of life in old age by delaying the onset of neurodegenerative conditions. Emerging evidence suggests that extracellular vesicles (EVs) secreted by human-induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) carrying a cargo of bioactive microRNAs (miRNAs) and proteins have the potential to regulate neuroinflammation, mTOR signaling, and autophagy. This study investigated whether intranasal hiPSC-NSC-EVs treatment at early middle age (12 months) and/or late middle age (16 months) could delay or prevent cognitive impairments associated with aging by modulating neuroinflammation, mTOR activity, and autophagic processes. To evaluate age-related outcomes, male and female C57BL/6 mice were administered either vehicle or hiPSC-NSC-EVs (10×10^9 EVs) intranasally at 12 months of age or 12 and 16 months of age. To evaluate brain domain-specific cognitive functions, animals were subjected to a series of neurobehavioral tests examining the integrity of the hippocampus, medial prefrontal cortex, and perirhinal cortex, including delayed alternation Y-maze, pattern separation, and temporal pattern processing tests.

Treatment with hiPSC-NSC-EVs at early or both early and late middle age resulted in improved cognitive performance across all behavioral tasks compared to vehicle-treated animals in both male and female mice. Improved cognitive function in the hiPSC-NSC-EVs-treated groups was associated with reduced astrocyte hypertrophy and microglial clusters, as well as microglia exhibiting the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome complexes in multiple brain regions, including the medial prefrontal cortex, hippocampus, and entorhinal cortex. Furthermore, the hiPSC-NSC-EVs-treated groups also exhibited diminished mTOR signaling and increased autophagy in multiple brain regions alongside enhanced hippocampal neurogenesis. Thus, IN administrations of hiPSC-NSC-EVs in early middle age could be an effective treatment to prevent age-related cognitive impairments associated with moderate neuroinflammation in both sexes.

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Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

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Topic: C.01. Brain Wellness and Aging

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BrightFocus foundation
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NIH F30EY035146

Title: Rescue of Age-related Vision Decline: Targeting ELOVL2-Mediated Fatty Acid Elongation

Authors: *D. SKOWRONSKA KRAWCZYK;
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Abstract: The retina is uniquely enriched in polyunsaturated fatty acids (PUFAs), which are primarily localized in cell membranes, where they govern membrane biophysical properties such as diffusion, permeability, domain formation, and curvature generation. During aging, alterations in lipid metabolism lead to reduced content of very long-chain PUFAs (VLC-PUFAs) in the retina, and this decline is associated with normal age-related visual decline and pathological age-related macular degeneration (AMD). *ELOVL2* (Elongation of very-long-chain fatty acids-like 2)

encodes a transmembrane protein that produces precursors to docosahexaenoic acid (DHA) and VLC-PUFAs, and the methylation level of its promoter is currently one of the best predictors of chronological age. Here, we show that mice lacking ELOVL2-specific enzymatic activity have impaired contrast sensitivity and slower rod response recovery following bright light exposure. Short-term and long-term intravitreal supplementation with the direct product of ELOVL2, 24:5n-3, in aged animals significantly improved visual function and reduced accumulation of ApoE and C3d in sub-RPE deposits. At the molecular level, the gene expression pattern observed in retinas supplemented with 24:5n-3 exhibited a partial rejuvenation profile, including decreased expression of aging-related genes and a transcriptomic signature of younger retina. Finally, we present the first human genetic data showing significant association of two variants in the *ELOVL2* locus with the onset of intermediate AMD, underlying the translational significance of our findings. In sum, our study identifies novel therapeutic opportunities and defines ELOVL2 as a promising target for interventions aimed at preventing age-related vision loss and other age-related central nervous system conditions.

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Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.21/Web Only

Topic: C.01. Brain Wellness and Aging

Title: Visualization of protective effect against tubulin aggregation in vitro and neuronal cells by molecular chaperone alphaB-crystallin, which supports human daily living

Authors: *Y. ATOMI[1], S. HORINOUCHI[1,2], M. SHIMIZU[1], D. INOUE[3], Y. FUJITA[4], E. FUJITA[5], A. ATOMI[1,2], T. WATANABE[2];
[1]Teikyo University, Advanced Comprehensive Res. Organization, Tokyo, Japan; [2]Tokyo Univ. Agric. Technol., Tokyo, Japan; [3]Kyushu University, Fac. pf Design, Fukuoka, Japan; [4]Prevent Sci. Co. Ltd., Tokyo, Japan; [5]:Teikyo University, Fac. of Med. Technol., Tokyo, Japan

Abstract: A large number of studies have reported that αB-crystallin is expressed in neurodegenerative brains; however, it is also ubiquitously present in adhesive cells of mammalian organisms. Notably, it is highly expressed in oxidative, antigravitational slow-twitch and cardiac muscle cells, both of which support daily life by maintaining the dynamic instability of the cytoskeleton—particularly the tubulin/microtubule (MT) system—and by preserving viability through anti-apoptotic functions. Using αB-crystallin knockout mice, several studies

have shown that αB-crystallin ameliorates neurodegenerative conformational diseases and is the only heat shock protein (HSP) reported to extend the lifespan of polyQ-expressing mice (Hayashida et al., 2010). We have previously reported that αB-crystallin acts as a molecular chaperone for tubulin (Arai et al., 1997; Ohto-Fujita et al., 2007) and MTs (Fujita et al., 2004), enhancing resistance to nocodazole and calcium in C2C12 and C6 cells. The interaction is highly dynamic, especially in beating cardiomyocytes (Ohto et al., 2020). In earlier work, we used deletion mutants to show that the α-crystallin domain binds tubulin dimers, while the N-terminal one-third interacts with MAPs (2017). In the present study, we visualized αB-crystallin's protective effect on porcine brain-derived tubulin in vitro. Since turbidity assays cannot reflect actual tubulin aggregation, we tracked aggregation of fluorescently labeled tubulin at 37°C. When 10 μM tubulin was incubated for 12 hours, αB-crystallin suppressed turbidity in a concentration-dependent manner. Epifluorescence imaging after GMPCPP and taxol polymerization showed that tubulin aggregates formed after 0.5 hours without αB-crystallin, but were suppressed until 3 hours in the presence of 2.5 μM αB-crystallin. This suggests αB-crystallin delays tubulin aggregation, preserving MT polymerization. Interestingly, MT length significantly decreased in αB-crystallin RNAi-treated Neuro2A cells after 1 day. These findings support αB-crystallin's chaperone function in both striated muscle and neural cells, consistent with simulation models of αB-crystallin-tubulin interaction.

Disclosures: Y. Atomi: None. **S. Horinouchi:** None. **M. Shimizu:** None. **D. Inoue:** None. **Y. Fujita:** None. **E. Fujita:** None. **A. Atomi:** None. **T. Watanabe:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.22/D41

Topic: C.01. Brain Wellness and Aging

Support: NIGMS grant R35GM153232

Title: The α5-$GABA_A$ Receptor Positive Allosteric Modulator MP-III-022 Attenuates Surgery-Induced Cognitive Impairment via Microglial Modulation in Aged Mice

Authors: J. LYU[1,2], R. NAGARAJAN[1], J. ZHOU[1,2], M. WANG[1,2], M. KAMBALI[1], M. INOUE[1,2], *U. RUDOLPH[1,2,3];
[1]Comparative Biosci., [2]Neurosci. Program, [3]Carl R. Woese Inst. for Genomic Biol., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Postoperative neurocognitive disorder (poNCD), marked by a persistent cognitive decline following surgery, occurs primarily in the elderly. Age-related reductions in GABAergic signaling, especially via α5-containing $GABA_A$ receptors, may contribute to this vulnerability. We previously found that MP-III-022, a positive allosteric modulator selective for α5-$GABA_A$ receptors, prevents poNCD in aged mice. Here, we investigated the temporal mechanisms

underlying this protective effect. Using two mouse models for aging - chronologically aged mice and transgenic mice with partial ablation of dentate gyrus hilar somatostatin interneurons - we assessed whether MP-III-022 modulates early postoperative neuroimmune responses and/or reverses age-related cognitive deficits - which are potentially due to a reduced expression of α5-containing $GABA_A$ receptors - by enhancing the function of this $GABA_A$ receptor subtype several days after surgery. Laparotomy induced cognitive deficits in both models, as shown by impairments in Y-maze alternation, novel object recognition, Morris water maze, and contextual fear conditioning. MP-III-022 administration significantly attenuated these deficits, both when applied from 2 days before to 3 days after surgery and when applied only starting 4 days after surgery, suggesting that both mechanisms outlined above may be relevant. Immunohistochemical analysis revealed an increased pixel density with Iba1-staining, indicative of microglial activation. We are currently performing immunofluorescence staining to see whether IL-6 expression in the hippocampus is increased. The hypothesis that we are currently testing is that MP-III-022 treatment reduces $TMEM119^+/CD68^+/IL\text{-}6^+$ microglial responses. Flow cytometry studies are ongoing to examine whether there is a surgery-induced expansion of $CD11b^+/TMEM119^+$ microglia co-expressing CD68, IL-6, and MCP-1, and if this is the case, whether it is suppressed by MP-III-022. These findings support temporally distinct protective actions of MP-III-022 on attenuation of neuroinflammation in an early phase and cognitive enhancement in a late phase. Our results highlight the therapeutic potential of targeting α5-$GABA_A$ receptors to prevent or treat poNCD in an aging population.

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Poster

PSTR379: Aging: Therapeutic Strategies

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Topic: C.01. Brain Wellness and Aging

Support: Science and Engineering Research Board (SERB), Government of India (No. CRG/2020/004205)
Ramalingaswami Re-entry Fellowship, Department of Biotechnology, Government of India (No. BT/RLF/Re-entry/49/2018)

Title: Targeting CA2 perineuronal nets restores recognition memory and theta oscillations in aged mice

Authors: *S. F. MEHAK[1], A. GUPTA[2], G. GANGADHARAN[1];
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Abstract: Recognition of familiar versus novel stimuli is fundamental for survival and is often compromised in several neurodegenerative disorders where aging is a key factor. While components of the extracellular matrix (ECM), such as perineuronal nets (PNNs), have been implicated in memory maintenance, their mechanistic and behavioral contributions during aging remain unclear. Here, we employed a D-galactose-induced accelerated aging model in male C57BL/6J mice (*n* = 6 per group) to elucidate the causal link between hippocampal ECM dynamics and recognition memory during aging. Aged mice exhibited significant deficits in both social and object recognition memory, alongside increased *Wisteria floribunda agglutinin* (WFA) staining intensity of PNNs specifically in the dorsal CA2 (dCA2) hippocampal subregion. Local filed potential recordings revealed a reduction in theta power (3-7 Hz) in the dCA2 of aged mice compared to controls. To establish causality, PNNs were selectively degraded via chondroitinase ABC (ChABC) injections into the dCA2. ChABC treatment rescued recognition memory performance and restored theta oscillation power. These findings demonstrate that aberrant PNN accumulation in dCA2 contributes causally to age-related deficits in recognition memory and network dynamics. Targeting PNNs in this subregion may offer a novel therapeutic approach for age-associated cognitive decline.

Disclosures: S.F. Mehak: None. **A. Gupta:** None. **G. Gangadharan:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.24/D43

Topic: C.01. Brain Wellness and Aging

Title: Aging and pharmacological challenges: medication complexity in middle-aged and older adults

Authors: *P. J. GONZALEZ-CABRERA[1], B. BALAKRISHNAN[2], M. GANESAN[2];
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Abstract: Aging adults often face the challenge of managing multiple chronic conditions, leading to increased pharmacological regimen complexity. The Medication Regimen Complexity Index (MRCI) provides a structured assessment of medication burden by accounting for dosage forms, frequency, and specific instructions, offering insights beyond simple medication counts. This cross-sectional study compared MRCI scores between active community-dwelling late middle-aged adults (n = 12, 55.41 ± 5.80 years) and older adults (n = 15, 75.76 ± 8.03 years), and explored the relationship between medication complexity and balance confidence using the Activities-specific Balance Confidence (ABC) Scale. MRCI scores were significantly higher in older adults compared to middle-aged adults (4.37 ± 3.16 vs. 2.50 ± 4.98, p = 0.024). Furthermore, among older adults, MRCI was negatively correlated with ABC scores (Spearman's ρ = -0.406, p = 0.036), indicating that greater regimen complexity is associated with

lower self-reported balance confidence. These findings suggest that older adults with complex medication regimens may be at greater risk of balance-related functional limitations, potentially increasing fall risk. Clinical strategies that incorporate both pharmacological review and balance training may improve mobility outcomes in this population. Integrating MRCI assessments into routine evaluations may help clinicians identify and address dual contributors—medication burden and confidence—in promoting safe aging in place.

Disclosures: P.J. Gonzalez-Cabrera: None. **B. Balakrishnan:** None. **M. Ganesan:** None.

Poster

PSTR379: Aging: Therapeutic Strategies

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.25/D44

Topic: C.01. Brain Wellness and Aging

Title: Chronic administration of resveratrol attenuates age-related cellular damage in the hippocampus of Wistar rats.

Authors: *I. CESAR ARTEAGA[1], D. JUÁREZ SERRANO[1], A. NAVARRO CRUZ[1], A. CORTÉS-LOZADA[1], J. MORAN PERALES[2], A. DIAZ[3];
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Abstract: During aging, an organism undergoes a series of morphological, physiological, and biochemical changes. Brain aging is considered the main cause of cognitive decline and a risk factor for developing neurodegenerative disorders. Various theories have been proposed regarding aging, with one of the most studied being related to oxidative stress. In this context, the accumulation of free radicals promotes the progression of oxidative processes such as lipid peroxidation and the consequent damage to macromolecules.
To counteract this damage, the endogenous antioxidant system maintains homeostasis in the organism; however, with age, the effectiveness of this system decreases, requiring external support through preventive therapies such as exogenous antioxidants. Resveratrol is an important antioxidant that has demonstrated anti-aging properties. Therefore, the objective of this study was to evaluate the effect of chronic resveratrol administration on age-related cellular damage in the hippocampus of Wistar rats.
A total of 216 male Wistar rats, 3 months old, were randomly divided into three groups: control; vehicle (7.5% ethanol); and resveratrol (10 mg/kg body weight + 7.5% ethanol). The administration periods ranged from 2 to 18 months. Behavioral, biochemical, enzymatic, and histological tests were conducted. The results showed that chronic treatment with resveratrol at a dose of 10 mg/kg of body weight exerts a protective effect on hippocampal cells in male Wistar rats, as it improves lifespan, cognitive performance in short- and long-term memory, reduces oxidative molecules, and enhances antioxidant enzymatic activity. Additionally, a reduction in cellular damage and death was observed in the CA1, CA3, and DG subregions of the

hippocampus.
This study contributes to the growing understanding of resveratrol's role as a neuroprotective molecule capable of preventing the neurodegenerative process.

Disclosures: I. Cesar Arteaga: None. **D. Juárez Serrano:** None. **A. Navarro Cruz:** None. **A. Cortés-Lozada:** None. **J. Moran Perales:** None. **A. Diaz:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.01/D45

Topic: C.01. Brain Wellness and Aging

Title: Inflammaging in the Colon: Sex Differences and the Impact of Physical Exercise

Authors: *C. FRAHM[1], R. PETIT[2], M. HAASE[3], R. SIMON[3];
[2]Neurol., [1]Jena Univ. Hosp., Jena, Germany; [3]Univ. Hosp. Jena, Jena, Germany

Abstract: Inflammaging, a chronic low-grade immune activation, is well-established in the brain, with initial evidence suggesting a similar process occurs in the colon. While exercise can counteract inflammation, its impact on the aged colon is unclear. We analyzed colon tissue from male and female C57BL/6J/Ukj mice at ages 9, 15, 20, 24, and 30 months, including 20-month-old mice after 2 months of physical activity. Up to 35 pre-selected genes were tested to assess inflammaging and barrier function. Aging in males led to significant upregulation of cytokines and chemokines (Tnfα, Il6, Ccl3), glia-associated markers (Cd68, Itgam, P2rx7), and immune function markers (Vcam1, Icam1, Mmp7, Mmp9). In females, many of the same genes were significantly regulated (Il6, Il1ra, Il23a, Ccl2, Itgam, P2rx7, Vcam1, Icam1, Mmp7, Mmp9), but to a lesser extent. Females also showed greater downregulation of tight junction genes, particularly Muc2 and Cldn4. These findings indicate colon inflammaging in both sexes, with a stronger age-related pro-inflammatory profile in males and greater barrier dysfunction in females. We then tested whether physical activity could counteract these changes by integrating two paradigms: continuous (24/7) wheel access and running limited to 3 nights per week. In the colon, 24/7 activity significantly downregulated nearly all inflammatory- and glia-associated markers, with significant effects for *Ifnγ,* Il10, *Ccl2, S100b,* and *Iba1*. In contrast, the 3 night/week group showed a heterogeneous response, including non-significant increases of a few genes (e.g., *Il1ra*) and significant downregulation of *Ifnγ, Il1β, Ccl2*, lacking the clear anti-inflammatory profile observed with 24/7 activity. Behavioral testing in the same mice via Barnes Maze test revealed that 24/7 running improved cognitive parameters in training, short- and long-term memory, and cognitive flexibility, whereas the 3 night/week group showed no cognitive benefits. Our findings show that only continuous voluntary activity reduces colon inflammation and improves cognition in old male mice.

Disclosures: C. Frahm: None. **R. Petit:** None. **M. Haase:** None. **R. Simon:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.02/D46

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG064091
NIH Grant OD011133

Title: Microglial morphologies change during aging in common marmosets (Callithrix jacchus)

Authors: *K. A. PHILLIPS[1], S. K. BOYEN[1], K. X. OLIVEIRA[2], R. MEREDITH[3], C. SHERWOOD[2];
[1]Trinity Univ., San Antonio, TX; [2]George Washington Univ., Washington, DC; [3]Texas Biomed. Res. Inst., San Antonio, TX

Abstract: Microglia are critical modulators of neuronal activity, regulating brain function through removal of dying neurons, pruning non-functional synapses, and producing ligands that support neuronal survival. Microglial morphologies change with aging, shifting from a homeostatic, surveillant state (ramified morphology) to a more reactive or dystrophic state (amoeboid and dystrophic morphology). Dystrophic microglia are associated with senescence and chronic inflammation and are especially seen in very old brains and in neurodegenerative disease. Neuroinflammation, largely driven by activated microglia, has been identified as a crucial component of neurodegenerative diseases. Common marmosets (*Callithrix jacchus*) are a valuable translational nonhuman primate model for aging, and studies of neuroinflammation and neurodegenerative disease as they are small, genetically diverse platyrrhine primates; considered aged at 8 years and have a lifespan of 12+ years; and display age-related changes in motor, sensorimotor, and cognitive function. Here we quantified the proportion of microglial morphologies in 24 aged marmosets (7 - 18 years) in dorsolateral prefrontal cortex (dlPFC, Brodmann Area 9), hippocampal regions CA1 and CA3, and the entorhinal cortex (ENT) via immunohistochemistry against Iba-1 and stereological techniques. The proportion of ramified microglia in dlPFC, CA1, and CA3 significantly decreased with age, whereas the proportion of dystrophic microglia in all four regions significantly increased with age. Males displayed a significantly higher proportion of dystrophic microglia than females in CA1. Sex did not significantly affect the proportion of microglia morphologies in any other brain region. A significant interaction between age and sex was found for the proportion of intermediate microglia in ENT. These results support microglial dysfunction increases with aging in marmosets. Understanding the normal aging process, including changes in microglial morphologies, is critical in furthering marmosets as a model for neurological aging and neurodegeneration.

Disclosures: K.A. Phillips: None. **S.K. Boyen:** None. **K.X. Oliveira:** None. **R. Meredith:** None. **C. Sherwood:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.03/D47

Topic: C.01. Brain Wellness and Aging

Support: the Natural Science Foundation of China 32171085

Title: Alterations of structural-functional connectivity coupling in older adults with depressive symptoms

Authors: *T. LI, J. LIU, S. ZHAO, X. LI, Z. ZHANG;
State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China

Abstract: Prior research indicates that both structural and functional networks are compromised in older adults with depressive symptoms. However, the potential impact of abnormal interactions between brain structure and function remains unclear. In this study, we used multimodal neuroimaging data from 415 older adults with depressive symptoms and 415 age-matched normal controls, focusing on structural-functional connectivity coupling (SFC) as a key multimodal feature. We examined group differences in SFC and tracked its alterations in relation to symptom progression. Our results revealed that older adults with depressive symptoms showed significantly increased SFC in the ventral attention network compared with normal controls. Moreover, changes in SFC within the subcortical network, especially in the left amygdala, were closely linked to symptom progression. Subgroup analyses further revealed heterogeneity in SFC changes, with certain physiological health factors, such as metabolic diseases and sleep disorders, contributing to distinct neural mechanisms underlying depressive symptoms in this population. This study identifies alterations in SFC related to depressive symptoms in older adults, primarily within the ventral attention and subcortical networks. Subgroup analyses highlight the heterogeneous SFC changes associated with metabolic diseases and sleep disorders. These findings may serve as neurobiological markers for early symptom detection and enable more personalized interventions, ultimately improving the clinical management of depression in older adults.

Disclosures: T. Li: None. **J. Liu:** None. **S. Zhao:** None. **X. Li:** None. **Z. Zhang:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.04/D48

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01AG069196
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NIH Grant R01AG077541

Title: Neuronal and Synaptic Signatures of Age and Sex in the Anterior Olfactory Nucleus

Authors: *M. HU[1], Y. LI[2], S. LIU[2];
[1]Univ. of Georgia, Athens, GA; [2]Physiol. and Pharmacol., UGA Col. of Vet Med., Athens, GA

Abstract: The anterior olfactory nucleus (AON) is a imperative brain region with extensive reciprocal connections to the olfactory bulb and higher-order areas such as the piriform cortex and hippocampus. It plays essential roles in processing, modulation, and memory of olfactory input. Notably, the AON is among the first regions affected in Alzheimer's and Parkinson's diseases, two age-dependent and sex-biased neurodegenerative disorders with olfactory impairments as prodromal symptoms. These features suggest that AON function may be influenced by both age and sex. However, age- and sex-dependent variations in the intrinsic and synaptic properties of AON neurons remain poorly understood.Using whole-cell patch-clamp recordings in brain slices, we identified age- and sex-related differences in neuronal physiology in the AON of C57BL/6J mice. At the intrinsic level, both pyramidal neurons and interneurons in aged mice exhibited a depolarized resting membrane potential (RMP), while aged pyramidal neurons also showed elevated membrane resistance (Rm) compared to young counterparts, regardless of sex. In contrast, Rm in interneurons varied with both age and sex: aged males exhibited higher Rm than young males, while young females had higher Rm than young males. At the synaptic level, aging in male pyramidal neurons was associated with reduced excitatory and enhanced inhibitory input, reflected in decreased amplitude and frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and increased inhibitory currents (sIPSCs). In females, aging enhanced inhibitory activity, while effects on excitatory input were inconsistent. In interneurons, aging reduced sEPSCs and increased sIPSCs in both sexes. Additionally, sex differences were evident, with females showing reduced excitatory and increased inhibitory input compared to males. Post hoc analysis of AON neuron morphologies is ongoing with immunohistochemistry and confocal imaging.These findings revealed the age- and sex-dependent differences in the physiological function of AON neurons. To further investigate how these differences influence neuronal and circuit dynamics within the AON, future studies will employ in vivo multi-channel extracellular recordings in awake, head-fixed mice, preserving intact afferent and efferent pathways. Together, these in vitro and in vivo studies will lay the groundwork for uncovering the neurophysiological mechanisms underlying olfactory deficits associated with neurodegenerative diseases.

Disclosures: M. Hu: None. **Y. Li:** None. **S. Liu:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

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Topic: C.01. Brain Wellness and Aging

Support: NIH grants R01AG069196
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NIH grants R01AG077541

Title: Age- and Sex-Dependent Electrophysiological properties of Mitral Cells in the Mouse Olfactory Bulb

Authors: *D. ZHAO[1], C. VICKNAIR[1], Y. LI[2], S. LIU[2];
[1]Univ. of Georgia, Athens, GA; [2]Physiol. and Pharmacol., UGA Col. of Vet Med., Athens, GA

Abstract: Sex difference in olfaction and age-dependent olfactory decline have been well documented. However, the underlying mechanisms, particularly those beyond the level of sensory neurons, remain poorly understood. Notably, olfactory dysfunction is among the earliest symptoms of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease, both of which exhibit age dependency and sex bias. Therefore, understanding how aging and sex influence olfactory processing could enhance the utility of olfactory deficits as early and differential biomarker for the diagnosis of these diseases. The olfactory bulb (OB), which receives direct input from olfactory sensory neurons, serves as the initial site of synaptic processing of odorant signals via intricate neural circuits composed of excitatory projection neurons and inhibitory interneurons. While OB interneurons are continuously replenished through adult neurogenesis, projection neurons including mitral and tufted cells are long-lived and potentially vulnerable to cumulative age- and sex-dependent changes. Such changes may underlie the emergence of olfactory impairments associated with aging and biological sex. To investigate this, we evaluated odor detection performance in male and female C57BL/6J mice across young and aged groups and characterized the intrinsic neurophysiological properties of mitral cells (MCs), the principal output neurons of the OB. Behaviorally, aged mice exhibited reduced ability to detect food odors compared to young counterparts. Electrophysiologically, whole-cell patch-clamp recordings in OB slices revealed a depolarized resting membrane potential and elevated membrane resistance, and increased spontaneous firing rates in mitral cells from aged mice. Furthermore, we observed significant age-dependent alterations in several parameters of spontaneous and evoked action potentials, including afterhyperpolarization amplitude, threshold, duration, frequency, and amplitude, which collectively indicate enhanced excitability in aged MCs. Sex-related differences in MCs excitability were particularly pronounced in the aged cohort, with consistently greater excitability in MCs of female mice compared to aged-matched males. These findings highlight a combined influence of age and sex on the physiological function of OB mitral cells, advancing our ultimate goal of understanding how these biological variables interact to shape sensory processing in the OB in normal conditions and in the context of olfactory dysfunction associated with neurodegenerative disorders.

Disclosures: D. Zhao: None. **C. Vicknair:** None. **Y. Li:** None. **S. Liu:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.06/D50

Topic: C.01. Brain Wellness and Aging

Support: WuXi AppTec

Title: Vx-548: a selective nav1.8 inhibitor with broad-spectrum efficacy against acute pain and neuropathic pain

Authors: *J. TAN;
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Abstract: Acute pain and neuropathic pain are prevalent and severe conditions affecting 7%-20% of the global population, contributing to substantial societal and economic burdens. Although opioids remain a mainstay of treatment, concerns about misuse, addiction, overdose mortality, and limited efficacy in neuropathic pain highlight the urgent need for safer, non-opioid alternatives. The voltage-gated sodium channel NaV1.8, selectively expressed in peripheral nociceptive neurons of the dorsal root ganglia (DRG), has emerged as a promising target for pain modulation. VX-548 (Suzetrigine), a selective oral NaV1.8 inhibitor has recently been approved by FDA to treat moderate to severe acute pain in adults, highlighting the potential of this non-opioid analgesic to address other types of pain. In this study, we examined the pharmacokinetic (PK) properties and analgesic efficacy of VX-548 across multiple rodent models of acute and neuropathic pain to assess its translational relevance. Following oral administration, VX-548 exhibited dose-dependent PK profiles in both mice and rats. In the post-operative pain model in mice, VX-548 significantly alleviated mechanical allodynia, with peak effects observed at 2 hours post-dose. Mice with streptozotocin (STZ)-induced diabetic neuropathy, which typically exhibit mechanical allodynia, experienced significant relief following oral administration of 10 mg/kg and 30 mg/kg doses of VX-548. In the oxaliplatin-induced chemotherapy neuropathy (CIPN) model, a 30 mg/kg dose of VX-548 effectively attenuated mechanical allodynia. While in rats subjected to spinal nerve ligation (SNL), VX-548 produced a significant reduction in mechanical allodynia, starting at 1 hour post-dose, with effects lasting for up to 6 hours. In the postherpetic neuralgia model, VX-548 at a dose of 100 mg/kg was able to produce marked anti-allodynic responses. These results suggest that VX-548 produces robust, dose-dependent analgesic effects in diverse preclinical pain models, with particular efficacy in neuropathic settings.

Disclosures: J. Tan: None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.07/D51

Topic: C.01. Brain Wellness and Aging

Title: Resting-state alpha peak power and peak frequency alterations as eeg biomarkers in neurodegenerative diseases

Authors: *B. FEMIR-GÜRTUNA[1], B. SEVGILI[2,3], N. KAYA[2,3], I. CELIK[2], B. GÜNTEKIN[4,2];
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Abstract: Alterations in alpha frequency serve as critical biomarkers for neurodegenerative diseases, reflecting cognitive function and resting-state brain activity. The current study examined resting-state alpha peak power and resting-state alpha frequency differences in low (6-8 Hz) and high alpha (8-13 Hz) across 5 groups: Healthy Elderly (HE), Parkinson's Disease (PD), Parkinson's Disease with MCI (PD-MCI), Parkinson's Disease with Dementia (PD-D), and Dementia with Lewy Bodies (DLB) under resting-state eyes-open (EO) and eyes-closed (EC) conditions. Data were analyzed across 7 locations; Frontal (F3, F4), Central (C3, C4), Temporal (T7, T8), and Temporo-Parietal (TP7, TP8), Parietal-1 (P3, P4), Parietal-2 (P7, P8), Occipital (O1, O2) and electrodes were assigned to both hemispheres. Python was used to conduct non-parametric permutation tests to assess group differences across conditions and locations. Independent two-sided permutation tests with 10,000 resamples and a fixed random seed (random_state=42) were used to ensure reproducibility. For all multiple comparisons, FDR-corrected permutation was used. Statistics revealed significant power differences in 6-8 Hz, across Central, Parietal, Temporal, and Temporo-Parietal regions. DLB showed increased power compared to PD and HE, while HE showed less power than PD-D and PD-MCI. In 6-8 Hz peak frequency domain, DLB showed decreased frequency compared to PD-MCI, PD, and HE in Central, Parietal, Temporal, and Temporo-Parietal regions. Additional differences were observed between HE, PD, and PD-D, primarily in Parietal regions and under EC conditions. Significant 8-13 Hz power differences occurred under EC condition. Compared to HE and PD-MCI, DLB showed reduced power in the Occipital and Parietal regions. Peak frequency analysis showed that DLB differed from both HE and PD, particularly in the Central and Occipital areas, while PD-D differed from HE and PD in the Frontal and Occipital areas. Different effects were observed in eye conditions, showing both region and eye condition dependent changes. These findings suggest that alterations in power and frequency in alpha band differ across neurodegenerative conditions, offering potential biomarkers for disease differentiation and tracking the progression of the neurodegeneration.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.08/D52

Topic: C.01. Brain Wellness and Aging

Support: MCKNIGHT21003
SFI-AN-NC-AB-Independence Postdoctoral-0000744

Title: Extracellular matrix and microglial proteomic and histological signatures of cognitive aging in mice

Authors: *D. T. GRAY[1], A. GUTIERREZ[1], V. PANDEY[2], J. WOHLSCHLEGEL[1], R. A. MCDEVITT[3], L. M. DE BIASE[4];
[1]UCLA, Los Angeles, CA; [2]Biol. Chem., UCLA, LOS ANGELES, CA; [3]Comparative Med., NIA, Baltimore, MD; [4]Physiol., David Geffen Sch. of Med. At UCLA, Los Angeles, CA

Abstract: Healthy brain aging is not simply a lack of change in neurobiological function, but rather an active process that engages endogenous mechanisms of plasticity to protect network function despite cellular and molecular alterations that accompany aging. Even in the absence of overt pathology, synapse loss and changes in synapse plasticity are hallmark features of brain aging, and preserved synapse status is linked to better cognitive outcomes. Both microglia and the extracellular matrix (ECM) can regulate synapse function throughout development and in early adulthood and both of these neuron-extrinsic factors have been linked to cognitive abilities in aging animals. However, the ways in which microglia and the ECM interact to shape synapse and neuronal function in the aging brain are largely unknown. In this study we combine sophisticated behavioral-, imaging-, and proteomic approaches to identify microglial and ECM phenotypes that promote cognitive resilience in aging mice as well as those associated with cognitive decline. Using ECM-optimized proteomic workflows, we discovered striking regional differences in aging-induced ECM remodeling, resulting in excess ECM abundance that is aligned with synapse protein abundance across key basal ganglia nuclei. Using high-resolution imaging, we demonstrate that excess ECM deposition in the ventral tegmental area (VTA) of aging mice is tightly correlated with microglial aging phenotypes in this brain region. Moreover, VTA microglial and ECM status aligned with local excitatory synapse numbers as well as deficits in reward-based learning in aging mice. Finally, to further probe relationships between the ECM and cognitive aging outcomes, we used a battery of cognitive tests to classify young-adult and late-middle-aged mice and into average young, aging impaired, and aging unimpaired groups using unsupervised clustering approaches. The ECM from these animals was enriched using tissue fractionation approaches, and these samples underwent proteomic analysis. We identified numerous structural and regulatory ECM proteins, as well as microglial proteins, associated with the preservation of cognitive behaviors in aging mice. These include hyaluronan and proteoglycan link proteins (HAPLNs), serpin protease inhibitors, and the microglia-specific fractalkine receptor Cx3Cr1. Together, this study provides foundational observations that implicate microglia-ECM interactions in the regulation of synapse function and cognition across the lifespan.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant 20300-04262000-12200-61100-NIA00058

Title: Prevention of age-dependent decline in learning and memory by up-regulation of Synaptobrevin-2

Authors: *J. MILLER;
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Abstract: Vesicular Associated Membrane Protein 2 (VAMP2)/ Synaptobrevin 2 (syb2) regulates vesicular docking and fusion at the presynaptic terminal, making it essential for neurotransmission. Synaptobrevin-2 (syb2) has relevance to dementia in the elderly as syb2 levels decrease with age and selective reduction of syb2 causes cognitive impairment. It is also known that syb2 is highly expressed in the hippocampus and cerebral cortex, two important regions involved in learning and memory. We hypothesized that age-related decrease in syb2 levels is a key factor in cognitive impairment and a possible therapeutic target. To test whether maintaining syb2 levels in the aging brain improves learning and memory, we used a transgenic mouse strain (TgV2) that overexpresses syb2. We investigated the effects of enhanced syb2 levels on synaptic vesicular release using fluorescence microscopy, electrophysiology, and on spatial memory in behavioral assays. We obtained the following results: (1) In fluorescence imaging with FM dyes, TgV2 neurons showed a significantly faster neurotransmitter release, a greater number of vesicles in the recycling pool than WT neurons. (2) In whole-cell patch clamp, TgV2 neurons maintained synaptic transmission at high frequency stimulation. (3) Long-term potentiation (LTP) recordings indicated greater synaptic plasticity within the CA1 region of the aged hippocampus. (4) Severely aged (2-year-old) TgV2 mice maintained the capacity for spatial learning and memory in Morris water maze. Based on these and our previous results on syb2, we conclude that syb2 overexpressing mice can maintain cognitive function throughout the aging process by better resisting synaptic dysfunction leading to age-related cognitive decline.

Disclosures: J. Miller: None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: PSTR380.10/D54

Topic: C.01. Brain Wellness and Aging

Support: NIH R03AG072218
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NIH R01NS115707
NSF CAREER 1943906

Title: Age-related electrophysiological impairment of functional laminar connectivity in the mouse V1 and CA1 regions

Authors: *T. T. D. THAI[1], S. WELLMAN[4], N. SUEMATSU[5], T.-H. TUNG[2], C. GARCIA PADILLA[1], S. SRIDHAR[6], F. CAMBI[2], T. D. KOZAI[3];
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Abstract: Aging has been linked with widespread changes in neural circuitry, but its impact on laminar-specific connectivity remains poorly understood. Interlaminar communication is critical for regulating the structured flow of information and pinpointing disruption in the neural network. The primary visual cortex (V1) and hippocampal CA1 are key areas for visual processing and memory integration. We hypothesize that aging impairs functional laminar connectivity in mouse V1 and CA1. We performed weekly electrophysiological recordings from 16-channel microelectrodes in young (2-month) and aged (13-month) wild-type mice to assess evoked neuronal activity. Analyzing spiking activity and local field potentials (LFPs) provides insight into individual neuron output and population-level dynamics within and across cortical and hippocampal regions. We found that aged mice had significantly reduced mean LFP power ($p = 0.0021$). To determine whether this reflected broader network disruption, we quantified changes in coherence (Δcoherence), defined as the difference in LFP coherence between visually evoked and spontaneous states. Across frequency bands, aged animals had reduced Δcoherence compared to young mice. In the delta range (2-4Hz), Δcoherence was significantly lower between L4-L1/2/3 ($p = 1.13e-5$), L1/2/3-L5/6 ($p = 4.98e-11$), and L5/6-CA1 ($p = 0.0016$). Similarly, in the alpha-beta band, age effects were observed from L4-L1/2/3 ($p = 1.24e-11$) and L1/2/3-L5/6 ($p = 4.00e-9$). In the gamma range, Δcoherence between L1/2/3-L5/6 was reduced in aged mice (-0.0074 ± 0.010 Hz/Hz) compared to young mice (0.0089 ± 0.0089 Hz/Hz (group effect: $p = 0.0327$)). These indicate that functional laminar connectivity in V1 to CA1 regions declines with age. To further investigate how aging impacts the strength of neural connections, we calculated the phase-amplitude coupling modulation index (MI) using the Hilbert transformation of LFP power. A higher MI value represents a stronger coupling between LFP low frequency phases and high frequency amplitudes. The MI between young and aged animals was comparable, indicating that the cross-frequency coupling is preserved in normal aging. Further, we found that spiking activity metrics of single-unit yield and signal-to-noise firing rate ratio were not affected by ($N = 6$; yield: $p = 0.1064$; mean SNFRR over time: 0.714 ± 0.036

Hz/Hz vs 0.743 ± 0.034 Hz/Hz, p = 0.4154). These results suggest that aging impacts functional laminar connectivity with minimal effects on individual neuronal spiking. This research may help differentiate normal aging from early pathological changes associated with neurological diseases.

Disclosures: T.T.D. Thai: None. **S. Wellman:** None. **N. Suematsu:** None. **T. Tung:** None. **C. Garcia Padilla:** None. **S. Sridhar:** None. **F. Cambi:** None. **T.D. Kozai:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.11/D55

Topic: C.01. Brain Wellness and Aging

Support: Fondecyt 1231556
ANID Doctoral Fellowship 21221779

Title: Differential dopaminergic vulnerability and mitochondrial alterations over aging in Drosophila melanogaster

Authors: *P. AMADO-HINOJOSA, A. CHAMORRO-GONZÁLEZ, J. M. CAMPUSANO; Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: Aging involves progressive cellular and molecular damage, leading to tissue dysfunction. Dopaminergic neurons are strongly associated with age-related neurodegenerative diseases, including Parkinson's disease, and their impairment over aging has been associated with alterations in motor control. Notably, dopaminergic subpopulations show differential vulnerability, highlighting the complexity of their response to aging and pathology. Mitochondrial dysfunction is a hallmark of aging and neurodegeneration. Their spatial organization critically impacts function, and modulating mitochondrial dynamics has been shown to influence survival in various animal models. *Drosophila melanogaster* is a powerful model for studying neuronal aging, allowing for the simultaneous analysis of all dopaminergic clusters in an intact brain. Specific dopaminergic clusters have been shown to be selectively vulnerable in fly models for neurodegenerative diseases. We examined whether age-related changes in locomotor performance are associated with dopaminergic cluster-specific neurodegeneration and mitochondrial fluorescent signal, to assess if mitochondrial features may contribute to their differential vulnerability. The exponential phase of fly mortality began after 50 days post-eclosion, with females living up to 72 days and males up to 62 days. Basal locomotor activity declined at 30 days in both sexes, and their climbing ability significantly decreased by day 40 in males and day 30 in females. Notably, females walked significantly less than males until 30 days of age. We used a fluorescent mitochondrial marker to assess the presence and distribution of mitochondria in dopaminergic neurons over time. We observed an overall decrease in dopaminergic neuron number across aging. However, only the PPM3 cluster

exhibited significant and cluster-specific neurodegeneration. These neurons, which project to the central complex - a brain region involved in motor control- also showed a marked reduction in somatic mitochondrial fluorescence at 60 days. Despite overall neuron loss, total dopamine levels increased with age. Our findings suggest that mitochondrial parameters are differentially altered across dopaminergic clusters during aging, potentially contributing to their distinct susceptibility and underlying effects on dopaminergic-related behaviors like motor control. Future experiments manipulating mitochondrial spatial distribution will help to determine whether these structural changes have a causal role in dopaminergic vulnerability and motor decline. Supported by Fondecyt 1231556 (JMC) and ANID Doctoral Fellowship 21221779 (PAH)

Disclosures: P. Amado-Hinojosa: None. **A. Chamorro-González:** None. **J.M. Campusano:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.12/D56

Topic: C.01. Brain Wellness and Aging

Support: R01 MH119185
R01 MH120174
R56 AG066656
R01 MH119219
R01 MH107703
1P41EB029460

Title: Mapping the spatial correlation between glutamate expression and age-related volume loss using GluCEST and neurotransmitter receptor data

Authors: *J. STIFELMAN[1], M. K. PECSOK[1], A. ATKINS[1], C. MASTRACCHIO[1], H. ROBINSON[4], P. FREEDMAN[1], G. SHAFIEI[1], D. A. WOLK[2], R. C. GUR[1], R. T. SHINOHARA[3], D. R. ROALF[1];
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Abstract: Normal aging involves widespread cerebral volume loss, but how chemoarchitecture contributes to this process remains poorly characterized. While glutamatergic dysregulation has been implicated in brain aging, prior ^{1}H-MRS studies lack spectral resolution and spatial coverage. To address this, we used normative PET-derived NMDA, mGluR5, and $GABA_A$ receptor distribution maps, in vivo Glutamate Chemical Exchange Saturation Transfer (GluCEST) imaging, and structural MRI to test whether brain regions with pronounced age-

related volumetric loss colocalize with higher glutamate receptor density and GluCEST signal. Forty-eight healthy young adults (HYA; age: 23.2 [4.2] years; 52% female) and 36 healthy old adults (HOA; age: 72.2 [4.0] years; 64% female) underwent 3T MRI. ROI volumes were extracted using FreeSurfer (Desikan-Killiany and aseg atlases), and ROI-wise linear regression quantified group differences. Standardized betas indexed volume loss. Normalized receptor densities from the Neuromaps PET database were parcellated with the same atlases. Spatial colocalization was assessed by modeling receptor density as a function of the absolute standardized volume loss, weighted by -log(p) of group effects. Across cortical ROIs, lower NMDA receptor density was associated with greater volume loss (B = -0.90, p = .028); no associations were observed for mGluR5 or $GABA_A$. In subcortical ROIs, higher NMDA (B = 1.43, p = .034) and mGluR5 (B = 1.90, p = .012) density were associated with volume loss; no significant association was found for $GABA_A$. A separate HYA cohort (n = 34, including 22 participants from the volume loss analysis cohort; age = 21.3 [2.8] years; 44% female) underwent 7T GluCEST imaging. GluCEST values were parcellated to the same ROIs, and linear regression tested spatial associations with volume loss observed in the HYA versus HOA comparison described above. In cortical ROIs, greater volume loss was associated with higher GluCEST signal (B = 46.8, p = .002); no significant relationship was found in subcortical ROIs (B = -10.2, p = .629). Together, these findings suggest that cortical volume loss is linked to lower NMDA receptor density and higher GluCEST signal, suggesting that NMDA-mediated Glu signaling and Glu concentrations contribute differentially to volume loss. In subcortical regions, volume loss correlates with higher NMDA and mGluR5 density. $GABA_A$ density was not associated with volume loss in either region. These findings highlight a potential link between age-related volumetric changes and glutamatergic system dynamics, underscoring the need for further study of chemoarchitecture in age-related morphometric decline.

Disclosures: J. Stifelman: None. **M.K. Pecsok:** None. **A. Atkins:** None. **C. Mastracchio:** None. **H. Robinson:** None. **P. Freedman:** None. **G. Shafiei:** None. **D.A. Wolk:** None. **R.C. Gur:** None. **R.T. Shinohara:** None. **D.R. Roalf:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.13/D57

Topic: C.01. Brain Wellness and Aging

Support: R01 AG 079957
RF1 AG 082201

Title: Genome Wide Association Study Of Local Brain Age Gap Reveals Cellular Pathways Involved In Cortical Senescence

Authors: *A. MISHRA[1], N. KIM[1], N. CHOWDHURY[1], A. IRIMIA[2];
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Abstract: The brain age gap (BAG)—the difference between predicted and chronological brain age—is a promising biomarker for neurodegenerative diseases. While previous studies have explored the genetics of global BAG, growing evidence shows that brain regions age at different rates. Relying solely on global BAG may overlook critical localized changes, whereas regional brain age estimates better capture disease-relevant neurobiological variation. Genome-wide association studies (GWASs) identify single-nucleotide polymorphisms (SNPs) linked to specific traits across the genome. Prior GWAS of global BAG have found heritable loci associated with brain aging, Alzheimer's disease, and Type 2 diabetes. To our knowledge, this study presents the first GWAS of local brain age gap (LAG), uncovering SNPs linked to region-specific aging patterns throughout the brain. We used UK Biobank data from 41,859 cognitively normal individuals (21,983 females aged 45-83 yrs). A deep learning model was trained to predict voxel-level brain age from T1-weighted MRI scans. LAG was calculated by subtracting chronological age from predicted local brain age at each voxel and mapped onto 148 cortical regions using the FreeSurfer Destrieux atlas. Linear regression tested associations between 662,971 SNPs and LAG in each region. Genome-wide significance was defined as Benjamini-Hochberg FDR-corrected $p < 0.05$. We identified 1,212 SNPs significantly associated with LAG in at least one region. The paracentral lobule and sulcus had the most associations; the lateral orbital sulcus and occipital pole the fewest. Genetic overlap was strongest between bilateral homologous regions and within frontal, parietal, and limbic lobes. Notable SNPs included variants in KCNK2 (rs864736, rs59084003), GMNC (rs13089287), and MSL2 (rs1393786), all showing widespread positive associations with LAG and enrichment for pathways related to hormone biosynthesis and chondrocyte differentiation, suggesting these processes are involved in faster regional brain aging. In contrast, rs12146713 was associated with decreased LAG across broad cortical areas. This SNP maps to NUAK1, a gene regulating cytoskeletal organization and protein modification. This suggests that pathways supporting cellular stability and repair may slow regional brain aging. These findings offer new insights into the regional nature of brain aging and its underlying genetic architecture, paving the way for studies into the genetic drivers of age-related decline. Future research will investigate how these pathways shape region-specific brain aging and contribute to the onset and progression of neurodegeneration.

Disclosures: A. Mishra: None. **N. Kim:** None. **N. Chowdhury:** None. **A. Irimia:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.14/D58

Topic: C.01. Brain Wellness and Aging

Support: Internal department funding

Title: Biomarkers of neurodegeneration revealed through intracranial EEG

Authors: *H. ALIYARI[1], B. C. LEGA[2];
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Abstract: Understanding how neural circuit dynamics reflect early markers of neurodegeneration is essential for improving diagnostic and therapeutic strategies for Alzheimer's disease. Motivated by serum biomarker profiles—particularly the Aβ42/Aβ40 ratio, a well-established peripheral indicator of cerebral amyloid pathology—we examined whether hippocampal oscillatory activity and network-level connectivity derived from intracranial EEG (iEEG) could capture early signs of circuit dysfunction during episodic memory processing.Our analysis followed a two-step approach. First, we conducted a correlation analysis in a smaller subset of 13 participants with available biomarker data to identify associations between Aβ42/Aβ40 ratios and frequency-specific neural activity. This initial analysis revealed significant correlations in the gamma band—specifically, low gamma power in the parahippocampal cortex and high gamma power in the posterior hippocampus. Guided by these findings, we then extended our analysis to a larger cohort of 72 participants, focusing on gamma-band oscillations and connectivity across three medial temporal lobe subregions: the anterior hippocampus, posterior hippocampus, and parahippocampal cortex.We observed robust gamma-band connectivity in younger adults (under 45 years old), particularly between the posterior hippocampus and parahippocampal cortex, as well as between the parahippocampal and anterior hippocampus. These connections were consistently weaker or absent in older participants (over 45 years old), a disruption evident during both encoding and retrieval phases of memory processing. Compared to oscillatory power alone or peripheral biomarkers, gamma-band connectivity more clearly differentiated age groups, underscoring its sensitivity as an indicator of network-level dysfunction.Altogether, these results suggest that gamma-band connectivity may serve as a promising neural marker for early neurodegeneration. The parahippocampal cortex appears central in mediating interactions along the hippocampal axis, and its breakdown could signal the onset of pathological changes in memory-related circuits. These findings support the utility of EEG-based biomarkers for detecting large-scale network alterations associated with aging and Alzheimer's disease progression.

Disclosures: H. Aliyari: None. **B.C. Lega:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.15/D59

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant 1 R01 AG070913-01

Title: Variation in whole brain connectomes with age: magnetic resonance histology in 13 strains of mice

Authors: *K. J. HORNBURG[1], D. G. ASHBROOK[4,5], R. AZRAK[1,2], J. J. COOK[1], Y. QI[1], L. E. WHITE[2], R. W. WILLIAMS[4], G. A. JOHNSON[1,3];
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Abstract: We used magnetic resonance histology (MRH) with diffusion tensor imaging (DTI), to study age-related neuroanatomical changes in brain structure, including whole-brain connectomes, across 13 strains from the BXD family. We compared young adult brains at ~120 days (~14% of lifespan, $N = 47$) with strain and sex-matched old mice at 400-550 days (~64% of lifespan and post-reproductive, $N = 60$). The voxel volume (spatial resolution) for these images was 9×10^{-5} mm^3—more than 20,000 times smaller than that of the Human Connectome Project. Here we evaluate how age and sex in a genetically diverse cohort influences volumes of 231 regions of interest, the scalar DTI metrics of these ROIs, and their dMRI-derived connection strengths. With this cohort, the volumes of most neocortical ROIs and cerebellar vermis decrease with age, while the olfactory bulbs, most subcortical gray matter structures, and most white matter (WM) structures increase in volumes. Similarly, there was a general decrease in fractional anisotropy (FA) with age in many areas of the neocortex, and an increase in FA in WM. However, axial diffusivity (AD), radial diffusivity (RD), and mean diffusivity (MD) all decrease with age in most ROIs. There were evident differences in age-related effects for the 13 strains. Measurable FA changes varied widely with limited change in BXD34, BXD60, and BXD101, and widespread FA increases in BXD65b. Tractography was performed in DSI Studio and the strength of edges interconnecting nodes was assessed using the new Duke Mouse Brain Atlas (DOI: 10.1126/sciadv.adq8089). Dimensional reduction of the connectomes defined significant age-associated changes for multiple nodes. Visualization of the latent coordinates in multidimensional scaling spaces highlighted similarities and differences. As with volumes and scalar DTI metrics, these age-related changes varied greatly between strains, with age-effects being much more prominent in BXD29 than in BXD34. Age-associated changes in edge strength also varied greatly across strains. For example, edge strengths between the anterior cingulate cortex and the somatosensory cortex increased with age in BXD29 and BXD65b, but decreased in BXD60 and BXD101. Taken together, these data represent the largest preclinical assessment of age-associated change in brain structure with the highest resolution MRH data yet published. We demonstrate significant change in structure size, diffusion characteristics, and whole-brain connectivity, and, moreover, that genetic background is a key factor to brain aging.

Disclosures: K.J. Hornburg: A. Employment/Salary (full or part-time): Duke Center for In Vivo Microscopy, Radiology, Duke University. **D.G. Ashbrook:** A. Employment/Salary (full or part-time): University of Tennessee Health Science Center, University of Edinburgh. **R. Azrak:** None. **J.J. Cook:** A. Employment/Salary (full or part-time): Duke Center for In Vivo Microscopy, Radiology, Duke University. **Y. Qi:** A. Employment/Salary (full or part-time): Duke Center for In Vivo Microscopy, Radiology, Duke University. **L.E. White:** A. Employment/Salary (full or part-time): Duke Institute for Brain Sciences, Duke University. **R.W. Williams:** A. Employment/Salary (full or part-time): University of Tennessee Health Science Center. **G.A. Johnson:** A. Employment/Salary (full or part-time): Duke University.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.16/D60

Topic: C.01. Brain Wellness and Aging

Support: Next Generation EU [DM 1557 11.10.2022

Title: How Cognitive Reserve Shapes the Aging Brain: Toward a Neurofunctional profile

Authors: *B. MONACHESI[1], E. PISANU[3], S. FORNARO[2], S. LUCIA[2], R. RUMIATI[2]; [1]Scuola Internazionale Superiore di Studi Avanzati - SISSA, rome, Italy; [2]Scuola Internazionale Superiore di Studi Avanzati - SISSA, Trieste, Italy; [3]International School for Advanced Studies (SISSA), Trieste, Italy

Abstract: The cognitive reserve (CR) hypothesis suggests that individuals with higher CR can better cope with age-related changes, either by performing more effectively on cognitive tasks or by engaging more efficient and compensatory brain networks. CR is typically operationalized indirectly through life experiences such as educational attainment, occupational complexity, and engagement in leisure activities. These proxies can now be assessed using tools like the Cognitive Reserve Index questionnaire (CRIq), which provides a composite score. Despite the theoretical significance and potential of the CR framework, the relationships between CR proxy, cognitive performance, and brain activity remain unclear. A recent review on the topic highlights the need for new, comprehensive investigations. The present project aims to fill the gap and examine the impact of CR on cognitive changes known to occur with aging, particularly those affecting executive functions, spatial reasoning, and verbal fluency. To this end, we employed the n-back (1- and 2 back) task to assess working memory, a fluency task to assess verbal fluency, and a hand laterality task to assess motor imagery. Criq scores were used as a CR proxy. In a behavioral pilot study (N = 45, mean age = 70), we found that older adults with higher CR demonstrated better working memory performance, especially in more cognitively demanding conditions (i.e., 2-back), as well as enhanced verbal fluency. Additionally, they employed more efficient visual strategies during motor imagery. These tasks have been adapted for use in event-related functional magnetic resonance imaging (fMRI) paradigm to provide a neural description of the interplay between cognitive decline and reserve. We expect that individuals with higher CR and better cognitive performance will exhibit more efficient neural networks, particularly involving the prefrontal cortex and task-relevant regions, as well as compensatory mechanisms during more demanding conditions. This project may offer a deeper understanding of the factors that influence cognitive reserve (CR) and how they interact with both neural and cognitive components. The findings could inform the development of clinical and rehabilitative interventions aimed at preventing or mitigating cognitive decline and impairment.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.17/D61

Topic: C.01. Brain Wellness and Aging

Support: James S. McDonnell Foundation
NIH Grant R01 AG-063930

Title: Age-related dedifferentiation of task-activity is linked to the reorganization of large-scale intrinsic brain networks

Authors: *M. A. PIPOLY[1,2], M. Y. CHAN[2,1], Z. ZHANG[2,1], E. WINTER-NELSON[2,1], G. S. WIG[2,1];
[1]Sch. of Behavioural and Brain Sci., [2]Ctr. for Vital Longevity, Univ. of Texas at Dallas, Dallas, TX

Abstract: In humans, increased age is associated with alterations in task-evoked brain activity and large-scale brain network organization. Task-fMRI studies demonstrate that brain activity differs in both the magnitude and spatial pattern when comparing older relative to younger aged adults (typically discussed as a form of dedifferentiation). Parallel work examining large-scale brain networks has demonstrated that the modular organization of brain systems, measured during resting-wakefulness and goal-directed tasks, decreases with increased age. It is unclear whether and how these independent observations of aging are associated. We leveraged data from the Human Connectome Project-Aging (n=358, age=36-100) to examine whether the specificity of task-evoked brain activity relates to the modular organization of large-scale brain networks. Brain activity and functional connectivity were measured during three independent fMRI tasks (response inhibition, episodic memory encoding/retrieval, visual-motor processing) and during resting-wakefulness. Task specificity of brain activity was quantified based on a deviation in expected patterns of brain activation to a given task. Functional connectivity was used to assess system segregation, which quantifies the organization of large-scale modular brain networks. First, the specificity of task-evoked brain activity decreases with increased age, across all three tasks (response inhibition B=-0.001,p<.01; episodic memory encoding/retrieval B=-0.004,p<.001; visual-motor processing B=-0.021,p<.001). Second, task engagement is associated with a greater age-related decrease in system segregation relative to rest, with distinct alterations of functional connectivity characterizing each task. Finally, this task-related brain network re-organization varies in relation to individual differences in task-specificity across tasks, irrespective of age, such that task-related alterations in the functional connectivity of specific systems are closely related to alterations in task-specificity for each of the three tasks. Collectively, these findings indicate that age-related dedifferentiation of task-evoked activity is closely related to changes in task-related large-scale network organization across adulthood, thus bridging these prominent characteristics of adult aging.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.18/D62

Topic: C.01. Brain Wellness and Aging

Support: The Royal Society of New Zealand - Marsden Grant (20-UOA-045)

Title: Age and brain asymmetry: global stability with subtle regional shifts

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Abstract: Age-related changes in hemispheric structure and function may arise from the progressive recruitment of homotopic contralateral regions (decreased asymmetry), or from accelerated atrophy in one hemisphere relative to the other (increased asymmetry). This study examined how brain asymmetry changes with age in a large sample of 1,725 healthy adults aged 48 to 80 years (mean age = 62.1 years) from the UK Biobank. The T1-weighted brain images were processed using the CAT12 toolbox (https://neuro-jena.github.io/cat/) extracting 27 brain regions in each hemisphere as per the LPBA40 atlas (https://www.loni.usc.edu/research/atlases). After calculating the absolute asymmetry index for each brain region, we tested whether asymmetry correlated with age and whether this relationship was influenced by total intracranial volume (TIV) and biological sex. Statistical significance was established through 10,000-permutation Monte Carlo simulations controlling the family-wise error. Significant age-related effects were found in three regions. The parahippocampal gyrus and putamen showed positive correlations with age ($r = 0.10$, $p = 0.002$; $r = 0.08$, $p = 0.032$), indicating enhanced asymmetry in older individuals. In contrast, the cingulate gyrus showed a negative correlation with age ($r = 0.08$, $p = 0.013$), suggesting reduced asymmetry in older individuals. No significant moderating effects were observed for TIV or sex. Thus, overall, asymmetry seems to remain relatively stable across most brain regions, with only three of the 27 regions showing significant age-related changes. These findings suggest that the structure of the adult brain retains a largely consistent pattern of hemispheric asymmetry over time, at least in terms of volumetric differences. Future research may benefit from incorporating other anatomical measures, conducting longitudinal assessments, and including diverse cognitive outcomes to better understand how age-related asymmetry patterns may relate to changes in cognitive function during aging.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

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Program #/Poster #: PSTR380.19/D63

Topic: C.01. Brain Wellness and Aging

Support: RS-2024-00408736
NRF-2020R1A2C2007954
NRF-2017R1A2B4002675

Title: Brain-driven interventions of age-associated muscle loss

Authors: *S. PARK[1], S. KIM[1,2], S. KIM, PHD[1,2];
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Abstract: Aging-associated decline in the nigrostriatal dopamine (DA) system, a key regulator of motor function, contributes to reduced locomotor activity and skeletal muscle loss. However, no direct evidence has been demonstrated that long-term preservation of dopaminergic neuronal function in the substantia nigra (SN) can prevent age-associated muscle loss. In this study, we targeted the SN to investigate whether enhancing its anti-aging capacity could counteract these deficits. We introduced sirtuin3 (SIRT3) gene—encoding the mitochondrial deacetylase Sirt3 well known for its anti-aging properties—into the SN of mice using an adeno-associated virus serotype 1 (AAV1) vector. Long-term overexpression of SIRT3 in the SN led to a marked preservation of DA signaling, improved mitochondrial function, and reduced expression of senescence markers. These cellular effects translate into significant protection against age-associated decline in both motor activity and skeletal muscle mass. These results suggest that enhancing neural activity in the nigrostriatal DA system through sustained expression of anti-aging molecules may provide an effective strategy to mitigate motor and muscular deterioration during aging. These findings were recently published in the May issue of Signal Transduction and Targeted Therapy.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

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Taiwan National Science and Technology Council Grant 107-2410-H-002-124

Title: How Age and Culture Shape the Brain's Response to Value-Based Decision Making Under Risk

Authors: *Y. TAN[1], C. LEE[1], L.-H. HUANG[1], C.-C. CHEN[2], R. MAIR[3], A. H. GUTCHESS[4], J. O. S. GOH[1];
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Abstract: Value-based decision making under risk varies by age and culture, but the influence of these interacting factors remains to be delineated. Using fMRI, our study investigated the neural correlates underlying risky value-based decision-making across 201 younger and older adults in the United States and Taiwan. Participants engaged in a lottery choice task, choosing between options with varying possibilities of winning (or losing) points or risk-free status quo. Results showed an effect of age on risk-taking and risk-aversion that was modulated by culture. When expected value (EV) was negative, older participants showed greater acceptances than younger participants, with older Taiwanese exhibiting the most risk-taking. When EV was moderately positive, American participants showed more risk-aversion than their same-age-group counterparts. When EV was highly positive, older Taiwanese adults expressed distinct risk-aversion compared to all other groups. Whole-brain analyses revealed greater neural sensitivity to value and uncertainty with age. Compared to younger adults, older adults evinced heightened neural modulation to linear increases in EV in bilateral medial frontal cortex, superior temporal gyrus, and left amygdala; to increasing probability in bilateral anterior cingulate cortex, medial and middle frontal gyri, and bilateral amygdala; and to uncertainty (which is peak at stake probability 0.5) in bilateral anterior cingulate cortex, medial frontal gyrus, anterior insula, and middle frontal gyrus. Moreover, Taiwanese showed greater neural sensitivity to linear increases in probability than Americans in bilateral middle cingulate cortex. These aforementioned regions showing greater age-related neural sensitivity are implicated in emotional value encoding and control, suggesting that older adults experienced greater emotional salience of anticipated outcomes, and engaged more cognitive regulation. Integrating with the decision behaviors across age and culture, we postulate that older Taiwanese approached lottery choices by discounting probabilistic losses and probabilistic gains during loss and gain contexts, respectively, a behavioral pattern that is present but less prominent in older Americans. Overall, age is associated with probability discounting in value-based decisions whilst living in Taiwanese cultural contexts adds to it.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.21/E1

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant U19AG073585

Title: White Matter Microstructural Indicators of Cognitive Function in Middle and Late Adulthood: Human Connectome Project Aging/Adult Aging Brain Connectome Cohort

Authors: *Y. ZHANG[1], Y. HONG[1], L. ANTONUCCI[1], G. VIJAYARAGHAVAN[1], B. M. ANCES[2], S. E. ARNOLD[3], S. Y. BOOKHEIMER[4], R. L. BUCKNER[7], C. CRUCHAGA[9], J. S. ELAM[10], M. F. GLASSER[11], D. G. GHAHREMANI[5], M. P. HARMS[12], H. LAVRETSKY[6], D. S. MARCUS[13], R. MAIR[8], T. NICHOLS[14], R. WELSH[6], E. S. YACOUB[15], D. H. SALAT[1]; [1]Massachusetts Gen. Hospital/Athinoula A. Martinos Ctr. For Biomed. Imaging, Charlestown, MA; [2]Neurol., Washington University, St. Louis, Saint Louis, MO; [3]Neurol., Massachusetts Gen. Hosp., Boston, MA; [4]Psychiatry and Biobehavioral Sci., [5]Dept. of Psychiatry and Biobehavioral Sci., [6]UCLA, Los Angeles, CA; [7]Psychology and Ctr. for Brain Sci., [8]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [9]Dept. of Psychiatry, Washington Univ. Sch. o, Saint Louis, MO; [10]Neurosci., Washington Univ. in St. Louis Sch. of Med., St. Louis, MO; [11]Dept. of Anat. & Neurobio., [12]Dept. of Psychiatry, [13]Washington Univ. Sch. of Med., Saint Louis, MO; [14]Nuffield Dept. of Population Hlth., Univ. of Oxford/Oxford Big Data Institue, Oxford, United Kingdom; [15]Univ. Minnesota, Minneapolis, MN

Abstract: White matter (WM) degradation is common in aging and Alzheimer's disease (AD), but its role in preserved or exceptional late-life cognition is unclear. This study explores whether such cognition is associated with resistance to WM decline, compensatory changes, or absence of preclinical AD-related WM patterns. Cross-sectional diffusion-weighted MRI data from 1,127 participants (aged 36-90+; 57.5% female) in the Human Connectome Project in Aging (HCP-A)/Adult Aging Brain Connectome (AABC) cohort were analyzed. Neurologically healthy participants were grouped into three age strata: midlife, young-old, and older adult. Memory, fluid intelligence (FIQ), and crystallized intelligence (CIQ) were assessed, with age- and sex-adjusted scores classifying high (top 25%) or typical (middle 50%) performers. Tract-based spatial statistics were used to generate voxel-wise FA maps. Higher cognitive performance was linked to greater FA across ages, with domain-specific trajectories. Memory-related FA differences between high and typical performers increased with age, while FIQ/CIQ associations weakened in later life. High performers showed elevated FA in the superior longitudinal fasciculus, corona radiata, and internal capsule. Memory and FIQ were linked to FA in the corpus callosum and splenium/cingulum, respectively. Effect sizes were largest between extreme groups. WM integrity is robustly associated with cognitive resilience, yet patterns vary by domain and age. Unlike AD-vulnerable tracts, resilience-linked WM spans broader sensorimotor and interhemispheric connectivity. Strengthening memory-WM links in older age suggests compensatory mechanisms, while diminishing FIQ/CIQ links may reflect differential development and/or aging trajectories. These cross-sectional results must be interpreted with

caution as cognitive and neural trajectories are likely more informative to capture baseline differences and track neurocognitive change. Future longitudinal analysis in the HCP-A/AABC cohort will clarify whether WM integrity predicts sustained resilience.

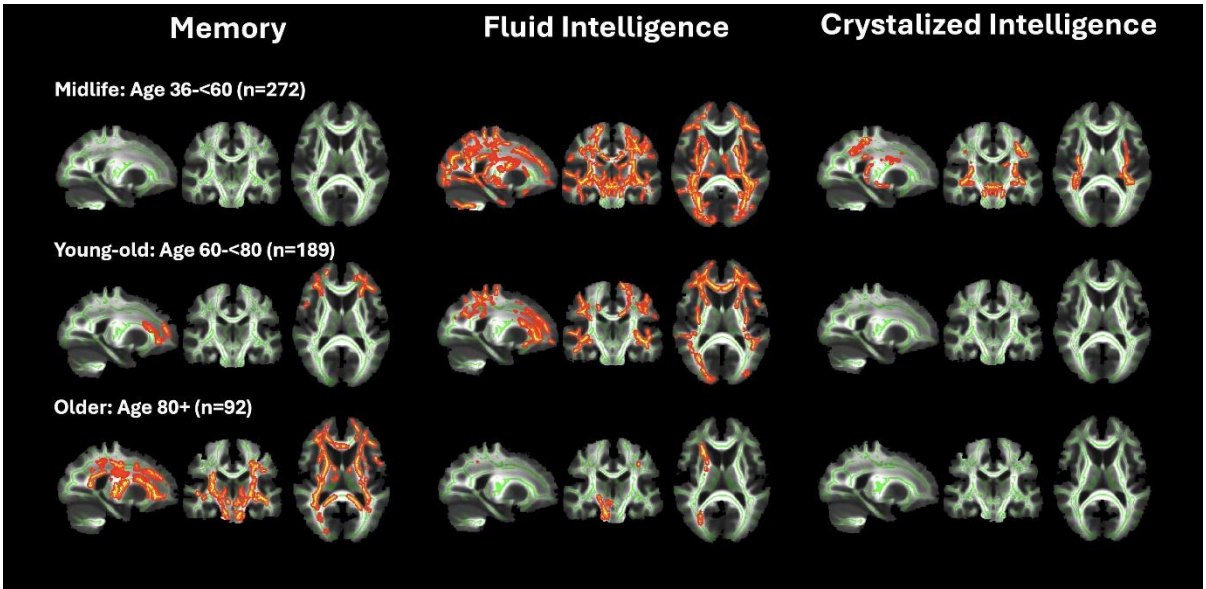

Figure 1. High vs. low performers by age group (36–<60, 60–<80, 80+) using a 25% threshold. Red-yellow indicates $p < 0.05$.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.22/E2

Topic: C.01. Brain Wellness and Aging

Support: by The University of Alabama through the University of Alabama College Academy of Research, Scholarship, and Creative Activity to I.M., and the University of Alabama, Birmingham/The National Institutes of Health, Grant/AwardNumber: P30AG031054

Title: Differential effect of sleep quality on resting-state networks across age and sex

Authors: *S. GOURABI[1], S. TAN[2], M. CRIBBET[2], J. CUNDIFF[2], I. MCDONOUGH[1,2]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Psychology, Univ. of Alabama, Tuscaloosa, AL

Abstract: Aging is often accompanied by decreased sleep quality, which can lead to cognitive decline and an increased risk of Alzheimer's disease and related dementias. Women often have more sleep disruptions than men, as well as higher prevalence rates of dementia. We do not yet understand how sleep disruptions in older women impact brain health related to cognitive decline and dementia risk. This study investigated how age and sex moderate the impact of sleep quality on resting-state functional connectivity (rsFC) within the Default Mode (DMN) and Salience Network (SN) and how those relationships were related to cognitive function. We analyzed rsFC and the Pittsburgh Sleep Quality Index (PSQI) scores in 95 adults (36 males, 59 females) aged 20 - 74 years. Results revealed a significant three-way interaction between age, sex, and global PSQI score on DMN connectivity with the left superior parietal lobule (SPL), part of the frontoparietal network (*cluster* $p_{FWE} = 0.046$, *peak* $p_{uncorr} < 0.000005$, $k=128$). This interaction was driven by females: poor sleep was linked to reduced connectivity in younger females and increased connectivity in older females (*left LP-SPL:* $\beta = 0.076$, $se = 0.019$, $p_{corr} = 0.00078$). A two-way interaction between age and global PSQI (*cluster* $p_{FWE} = 0.0019$, *peak* $p_{uncorr} < 0.00005$, $k=203$) was observed for SN connectivity with the right precentral/postcentral gyrus (Pre/PoCG), part of the sensorimotor network. Younger adults with poor sleep showed increased SN-sensorimotor connectivity, suggesting hyperarousal, while older adults showed decreased connectivity. Follow-up analyses indicated sleep latency was the largest contributor to the sleep quality-connectivity effects found for young adults, and sleep duration for older adults for the DMN-FPN, whereas for the SN, sleep latency was significant in younger adults and sleep medication was significant in older adults. No significant relationships were found using the amygdala as a seed region. Partial Least Squares Regression revealed altered DMN connectivity was associated with poorer spatial episodic memory ($p = 0.040$). These findings suggest that older women may be particularly vulnerable to sleep-related brain alterations that accelerate biological aging and episodic memory decline. The age-dependent patterns in the SN, particularly decreased connectivity in older adults with poor sleep, are inconsistent with a simple hyperarousal model for this age group and may align with network desegregation. Given higher Alzheimer's risk in women and the link between DMN disruption and cognitive decline, sleep may represent a key early intervention target.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.23/Web Only

Topic: C.01. Brain Wellness and Aging

Support: NIDCD T32 DC014435
R01 DC014467
R01 DC017619
P50 DC00422
VA BX006478
INBRE BIPP

Title: Aging Effects on Microglial Activation and Myelin Integrity in the Mouse Central Auditory System

Authors: *E. FABRIZIO-STOVER[1], T. JENKINS[1], J. BARTH[2], H. LANG[1];
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Abstract: Age-related auditory central gain in older adults is hypothesized to arise from the selective loss of GABAergic inhibition within the central nervous system (CNS). The loss of inhibition may result in a shift towards hyperexcitability, known as central gain. Age-related auditory central gain is associated with poor speech-in-noise understanding in older adults. The mechanisms underlying central gain, such as the underlying cause of loss of inhibition, are not well understood. One possible factor could be age-related inflammation, as there is increasing evidence that pro-inflammatory microglia can modulate cortical activity through modulation of GABAergic inhibition and myelination (e.g., via GABA receptors within the oligodendrocyte lineage). However, this has not been studied in the context of the aging auditory system. Here, we use immunohistochemistry of the auditory cortex (AC) and inferior colliculus (IC) in young (4M) and aged (2+Y) CBA/CaJ mice to characterize the associations between aged, activated microglia with GABAergic inhibition and myelin integrity. We found that aged mice had more and larger activated microglia compared to young mice in both the AC and IC. GAD 65/67 neuronal expression was reduced in older mice in both regions, but interestingly, a sub-set of aged microglia exhibited engulfment of GAD67+ cell processes or cellular debris. Furthermore, age-related changes in the expression of myelin basic protein (MBP+) were seen in in the AC. In C57BL/6 mice, another model of age-related hearing loss, enrichment analysis of bulk RNA sequencing data revealed that aging was associated with myelin dysregulation in the AC, but not as strongly in the IC. Together, these data demonstrate that aging may affect microglia activity and myelin integrity in multiple regions of the auditory system and that aged microglia may play a role in the modulation of GABAergic inhibition with age. Further studies are needed to investigate how age-related inflammation and aging microglia directly affect auditory functioning, as well as to investigate potential causal relationships between microglial activity, central gain and aging auditory phenotypes.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.24/E3

Topic: C.01. Brain Wellness and Aging

Title: Structure-function coupling using gray matter topography predicts cognitive change in fluid reasoning

Authors: *G. ARGIRIS[1], Y. STERN[1], C. G. HABECK[2];
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Abstract: Understanding the relationship between brain structure and function is important to elucidating the neural correlates of cognitive aging. In this study, we estimated structural connectivity (SC) by computing the Kullback-Leibler (KL) divergence between gray matter maps across pairs of brain regions, capturing regional similarity in gray matter topography using structural MRI data. Individual SC matrices were constructed using the Schaefer 400-parcellation atlas augmented with 19 subcortical regions, yielding a total of 419 regions of interest (ROIs). Functional connectivity (FC) was derived from resting-state fMRI using zero-lag Pearson correlations between time series of all ROI pairs. Structure-function coupling (SFC) was defined as the correlation between each node's SC and FC profiles, yielding 419 SFC values per subject.We applied this approach to 154 cognitively healthy older adults (aged 55+) from the Reference Ability Neural Network (RANN) cohort. Baseline structural and functional MRI data were used to compute SFC, while longitudinal neuropsychological assessments across two time points were used to characterize cognitive change. We examined whether baseline SFC could predict cognitive trajectories across four domains—Memory, Fluid Reasoning, Processing Speed, and Vocabulary—while adjusting for changes in brain integrity, operationalized as longitudinal variation in whole-brain cortical thickness (CT), in each model. Elastic net regression was used to identify the most predictive SFC features.Although global SFC (i.e., mean across nodes) did not predict cognitive change in any domain, regional SFC patterns were significantly associated with change in the Fluid Reasoning domain. Elastic net regression identified six predictive nodes—predominantly right-lateralized—including the right middle orbitofrontal cortex, right inferior frontal gyrus (pars triangularis), right caudate, right supplementary motor area, and left calcarine cortex. These regions collectively support the integration of executive control, flexible problem-solving, and attentional regulation, potentially underscoring the neural mechanisms that underlie fluid reasoning performance.Our findings demonstrate the value of SFC—even when derived from morphometric features such as gray matter topology—in identifying neural markers of cognitive reserve and supporting fluid reasoning in aging.

Disclosures: G. Argiris: None. **Y. Stern:** None. **C.G. Habeck:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.25/E4

Topic: C.01. Brain Wellness and Aging

Support: R24 AG065172
James S. McDonnell Foundation
K01 AG078407
P30 AG066518
P51 OD011092
R01 AG092219

Title: Age-related functional brain network decline in anesthetized rhesus macaques

Authors: *E. WINTER-NELSON[1], W. LIGUORE[3], A. GRIGORIAN[3], M. Y. CHAN[4], E. BERGMANN[5], G. VILL[6], L. HAN[1], Z. ZHANG[2], I. KAHN[7], B. E. RUSS[8], A. R. WEISS[9], G. S. WIG[2];
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Abstract: A hallmark of human aging is the reorganization of resting state functional correlation (RSFC) brain systems. The extent to which brain systems are functionally differentiated declines throughout adulthood and can be quantified using system segregation (Wig, 2017). Higher system segregation is associated with better cognitive ability in healthy adults (Chan et al., 2014) as well as patients with Alzheimer's Disease (Ewers et al., 2021; Zhang et al., 2023), while within-individual system segregation decline is prognostic of dementia severity in older age (Chan et al., 2021). However, the mechanisms underlying age-related system segregation changes are unclear. Animal models offer a means of gaining insight towards age-related brain network decline, but it remains uncertain whether comparable age-related RSFC brain network changes exist in other species, and how these changes compare to those observed in humans. Our previous work has demonstrated age-related decline in resting-state brain system segregation among mice (3-23 months old; Winter-Nelson et al., in revision) and marmosets (2-10 years old; Winter-Nelson et al., in prep.). Here, we examine age-related brain network alterations in the anesthetized rhesus macaque. Combining archival and newly acquired resting-state fMRI data from the Oregon National Primate Research Center (ONPRC), we built a large lifespan resting-state fMRI dataset spanning 4-32 years of age (n=112, n=60 females). Brain networks were built by first parcellating the brain into functional areas (nodes) and then detecting functional communities amongst the nodes. System segregation was calculated for the brain network of each individual animal. A linear mixed-effects model accounting for age, sex, and scanner effects revealed a main effect of age on system segregation ($F(1,101) = 4.862$, $p = .030$). This result affirms the use of macaque models of brain network aging and opens the door to subsequent investigations probing specific factors conferring resilience and vulnerability to age-related decline in multiple primate species.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.26/E5

Topic: C.01. Brain Wellness and Aging

Support: NIH R56-MH13048
Sloan Research Fellowship

Title: Reactivating Exploratory Learning in the Aging Brain

Authors: *S. M. CINQUEMANI, S. KORDE, Y. LEE, E. HWANG;
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Abstract: Exploratory behavior is critical for identifying optimal solutions during learning but declines with age in both humans and mice. We previously found that optogenetic stimulation of the posterior parietal cortex (PPC) in older mice restores both exploration and learning to youthful levels, implicating the role of the PPC in regulating exploratory learning. Here, we investigated the mechanisms driving age-related decline in exploratory learning and the basis for the restorative effects of PPC stimulation using optogenetics, in vivo two-photon imaging, and immediate early gene (IEG) expression quantification. First, we found that, unlike stimulation, PPC inactivation did not enhance exploration or learning in a sensory-guided decision-making task, suggesting that diminished PPC activity underlies age-related deficits in exploratory behavior and that stimulation counteracts this decline. Next, two-photon calcium imaging revealed that in younger mice, PPC neurons exhibited elevated pre-trial activity on exploratory trials, a signal absent in older mice, suggesting that trial-specific PPC engagement supports exploratory choices and declines with age. Finally, we found that older mice exhibit significantly reduced c-Fos expression in the PPC following task performance, indicating a broader age-related decrease in PPC activity. Together, these findings suggest that diminished PPC activity underlies the age-related decline in exploratory learning and highlight PPC activation as a potential therapeutic avenue to mitigate cognitive aging.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.27/E6

Topic: C.01. Brain Wellness and Aging

Support: K08 MH122893
R01 MH068542
RF1 AG080818
Hope for Depression Research Foundation (HDRF)

Title: Age-dependent changes in dentate gyrus serotonin during behavioral pattern separation

Authors: *S. KHAN[1,2], G. F. TURI[2], H. CHUNG[2], H. CHEN[2], R. HEN[2], W.-L. CHANG[2]; [1]New York State Psychiatric Institute/Columbia Univ. Irving Med. Ctr., Manhattan, NY; [2]Dept. of Psychiatry, Columbia Univ. and New York State Psychiatric Inst., New York, NY

Abstract: Pattern separation, the process of distinguishing similar experiences into distinct neural representations, shows marked decline with aging. This cognitive process is mediated by the dentate gyrus (DG), but we do not yet know how age-related changes in neural circuitry contribute to impaired discrimination ability. Additionally, the role of neuromodulators like serotonin on this circuit remain poorly understood. Serotonergic input to the DG influences plasticity and circuit function, and the broader serotonin system changes with aging. In the current study, we use fiber photometry and neurotransmitter sensors to monitor serotonin dynamics during fear learning and behavioral pattern separation in young (2 months, n=7) and aged (19 months, n=7) C57BL/6J mice. We injected AAV-GRAB-5HT bilaterally and implanted fiber optics in the dorsal DG. Biosensor activity was recorded during a 4-day fear learning and context discrimination task. Although aged mice demonstrated intact fear learning, young mice showed significantly different freezing between similar contexts by the second day of exposure ($p<0.01$), while aged mice were not able to discriminate between contexts. There was a gradual change in GRAB-5HT response to the footshock in young mice over successive days ($p<0.05$), which was not found in aged mice. We also found a trend towards age-related changes in DG serotonin dynamics to the initial encoding foot shock. This study demonstrates how serotonin modulation of the DG might contribute to age-related decline in behavioral pattern separation performance in mice. A comparison of these dorsal DG findings to ventral DG serotonin dynamics will also be presented. Ongoing work aims to further characterize the relationship between age-related DG serotonin dynamics and discriminative freezing behavior.

Disclosures: S. Khan: None. **G.F. Turi:** None. **H. Chung:** None. **H. Chen:** None. **R. Hen:** None. **W. Chang:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.28/E7

Topic: C.01. Brain Wellness and Aging

Support: R01AG029523 to B.R.
R01AG047972 to B.R. and H.L.

R01NS106711 to H.L.
R01NS106702 to H.L.

Title: Age-related differences in processing speed: default mode network and peripheral visual network connectivity during an fmri adapted digit symbol verification task

Authors: *M. ANDREO[1], G. SHERARD[2], Y. ZHAO[3], D. H. ABDELKARIM[4], B. P. RYPMA[5];
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Abstract: Age-related declines in processing speed have been hypothesized to underlie cascading effects on higher-order cognition associated with aging, such as executive function. Regions of the control and default mode networks (DMN), such as the dorsolateral prefrontal cortex, play a crucial role in managing increasing task demand in diverse aspects of cognition, including working memory. In this study, 48 young adults (M_{age} = 62.35) and 45 older adults (M_{age} = 24.31) completed dual-echo calibrated fMRI and phase-contrast ASL scans (22 transverse slices; flip angle = 90◦, TR = 4000 ms, TE1 = 13 ms, TE2 = 30 ms, voxel size 3.44×3.44×6 mm³, labeling duration = 1400 ms). In each trial, a "digit-symbol" key was displayed at the top of the screen with a single test digit-symbol pair below the key. Participants indicated by button press whether the test pair matched any of the key digit-symbol pairs. Trials lasted 4 seconds with a variable ITI (0, 4, 8, 12 s) and varying digit-symbol key set sizes (1, 3, 9). We examined age-related differences in brain function (Blood-oxygen-level-dependent (BOLD) signal from TE2 and functional connectivity) to predict slopes of reaction time across increasing set size demands during a scanner-adapted digit-symbol verification test (DSVT). BOLD signal was extracted from the Schaefer 2018 200 parcellation atlas. A regression analysis of set size-related RT slope on BOLD slope yielded significant ROIs from DMN, control, peripheral visual, and dorsal attention networks. In young adults, increased recruitment of the left dorsal prefrontal cortex was associated with reduced set size-related RT slopes. Young adults who recruited the left lateral prefrontal cortex and older adults who recruited the ventral prefrontal cortex and occipital pole displayed increased set size-related RT slopes. Using ROI-ROI age-group contrasts in connectivity during the trials with the highest demand (SS9), we observed that older adults showed greater positive connectivity than young in right and left precuneus-left temporoparietal lobe-left inferior parietal lobe networks. Older adults displayed greater negative connectivity than young in the left temporal occipital lobe-parietal occipital lobe-inferior parietal lobe-right occipital pole with bilateral medial prefrontal cortex-dorsal prefrontal cortex. Our results indexing the linear changes in BOLD signal across increasing set sizes suggest that recruitment of ROIs from the default mode network and peripheral visual network in older adults may display patterns of de-differentiation that could contribute to age-related slowing in processing speed.

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Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.29/E8

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG-036848
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AWARE
BvB Dallas

Title: Task-based fMRI changes in structure-function coupling of brain networks across the adult lifespan

Authors: ***L. T. NGUYEN**[1], E. E. PONGPIPAT[1], K. M. RODRIGUE[2], K. M. KENNEDY[3]; [1]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Dallas, TX; [2]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX; [3]Sch. of Behavioral and Brain Sci., The Univ. of Texas at Dallas, Dallas, TX

Abstract: In recent years, a growing body of research has explored the macroscale relationship between structural connectivity (SC) and functional connectivity (FC) across both healthy and clinical populations. Structural connectivity and functional connectivity coupling (SFC) quantifies how closely functional connectivity patterns align with the brain's underlying white matter architecture. This measure is crucial for understanding how the brain supports cognitive function across the lifespan and adapts to varying task demands. Previous work from our group examined changes in SC-functional relationships within the adult lifespan, focusing on specific regions such as the default mode and frontoparietal networks. In the present study, we investigated whether age modulates the effect of in-scanner task demands on SFC across seven canonical brain networks. We used cross-sectional data from 91 participants (mean age = 56.78 ± 18.21 years) drawn from the Dallas Area Longitudinal Lifespan Aging Study (DALLAS) cohort. During fMRI, participants engaged in multiple levels of an *n*-back working memory task (0-, 2-, 3-, and 4-back). For each participant, one structural adjacency matrix and four functional adjacency matrices (corresponding to each task condition) were constructed based on the Schaefer atlas with 400 parcellations. For each parcel, SFC was quantified as the Spearman's correlation between structural and functional connectivity matrices. These values were then averaged within the Yeo 7 networks. Linear mixed-effects models revealed that SFC increased with task difficulty ($F(1, 92) = 63.926$, $p < .001$) and also revealed a main effect of systems ($F(6, 2420) = 292.787$, $p < .001$). A significant age × system interaction also emerged ($F(6, 2420) = 19.781$, $p < .001$), indicating that the relationship between age and SFC varies by network system. These findings suggest that the coupling of structural connectivity and task-based functional connectivity does not remain constant across task difficulty nor network systems. With increasing working memory load, functional connectivity becomes increasingly more coupled to the structural infrastructure. This investigation underscores the importance of looking at task-based functional connectivity when studying structure-function relationships, since the age-sensitive brain is a dynamic structure that constantly changes in response to cognitive demands.

Disclosures: L.T. Nguyen: None. **E.E. Pongpipat:** None. **K.M. Rodrigue:** None. **K.M. Kennedy:** None.

Poster

PSTR380: Neurobiology of Aging: Neural Mechanisms and Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.30/E9

Topic: C.01. Brain Wellness and Aging

Support: The Phil & Penny Knight Initiative for Brain Resilience provided grant number KCG-116

Title: Reducing endocannabinoid degradation rejuvenates the aging brain

Authors: *C. L. PORTER;
Stanford Univ. Sch. of Med., Stanford, CA

Abstract: 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:115%; mso-pagination:widow-orphan; font-size:12.0pt; 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;} Chronic inflammation is a known pathological mechanism that underlies cognitive decline in the aging brain. We recently identified that peripheral inflammatory signaling via the lipid messenger prostaglandin E_2 (PGE_2) increases with age, while treatment that reduces myeloid PGE2 EP2 receptor signaling can prevent brain inflammation and restore cognitive function to a more youthful state. There is also an emerging understanding that the endocannabinoid signaling molecule, 2-arachidonic acid (2-AG), is a major substrate for the generation of PGE_2 in the brain and other organs. We therefore hypothesized that elevated endocannabinoid signaling in aging promotes increased PGE_2 generation, and that reducing 2-AG to PGE_2 metabolism would help reduce brain inflammation and improve cognitive function. Using 2-photon imaging of hippocampal neuronal activity in awake and behaving mice running on treadmills with cued belts, we find that calcium activity coupling to endocannabinoid release remains similar between young adult (3-6 months) and aged (21-24 months) animals. Furthermore, administration of the monoacylglycerol lipase (MAGL) antagonist JZL 184 (16 mg/kg, i.p.), which inhibits 2-AG hydrolysis and eventual conversion to PGE_2, was associated with enhanced and prolonged 2-AG transients across all groups. With chronic administration of JZL across 6 weeks (16 mg/kg, 3 times per week), spatial memory performance in a Barnes maze task was substantially improved in aged animals, returning to levels comparable to those in young mice. We are continuing to assess the impacts of chronic JZL treatment on tissue-specific PGE_2 expression, brain cytokine levels and

expression of inflammatory markers on microglia. Overall, we believe that targeting upstream mechanisms that link brain endocannabinoid signaling to inflammatory mechanisms has great potential to improve cognitive function in the aging brain.

Disclosures: C.L. Porter: None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.01/E10

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

Support: AG075341-01A1

Title: Peripheral inflammation alters microglial response to amyloid pathology and increases amyloid protein in the APP/PSEN1 model of Alzheimer's disease

Authors: *R. M. SPICER, A. A. TIENDA, F. E. HARRISON;
Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Severe sepsis and delirium are strong predictors for poor long-term cognitive decline, including the development of Alzheimer’s Disease and Related Dementias (ADRD). Understanding how peripheral inflammatory insult can influence persistent cognitive decline and ADRD progression is crucial to identifying at-risk individuals and guiding therapeutic strategies. In this study, 12-month-old APP/PSEN1 transgenic mice and wild-type littermates received intraperitoneal injection of polymicrobial Cecal Slurry (CS) to investigate changes to microglial number and morphology, and the progression of AD-associated pathology in response to sepsis. To determine how microglia respond to amyloid beta (Aβ) plaques formed prior to- and following CS-treatment, Methoxy-X04 (MX-04) and Thiazine Red (TR) were injected into the mice 24 hours and 30 days post-sepsis, respectively.Histological analysis and post-image analysis using IMARIS software confirmed that compared with wild-type littermates, APP/PSEN1 mice have significantly more microglia in the cortex and hippocampus. Microglia from APP/PSEN1 mice also show a more activated phenotype. Although number and morphology of microglia were similar regardless of CS-treatment, systemic inflammation drove clear differences in cell distribution relative to pathology. 3D reconstruction of MX-04$^+$ and TR$^+$ Aβ plaques showed that CS-treated APP/PSEN1 mice had significantly fewer microglia clustering around Aβ plaques in the dentate gyrus and elevated soluble Aβ protein compared in the hippocampus compared with vehicle-treated APP/PSEN1 mice.These findings suggest that systemic sepsis impairs microglial engagement with amyloid plaques, which may disrupt the mechanisms associated with Aβ clearance, thus exacerbating Aβ pathology. This work highlights the modulatory role of peripheral inflammation in progression of AD and requires further investigation to determine the behavioral implications and pathological changes in later stage disease progression.

Disclosures: R.M. Spicer: None. **A.A. Tienda:** None. **F.E. Harrison:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.02/E11

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

Support: PRIN 2022 PNRR Prot: P2022YW7BP, CUP:J53D23016130001

Title: Inflammation and oxidative stress as early triggers of neurodegeneration in mouse models of familial and sporadic Alzheimer's disease

Authors: *R. SOLLAZZO[1], D. D. LI PUMA[1,2], M. RINAUDO[1,2], F. PACIELLO[1,2], S. AUTELITANO[1], G. ACETO[1,2], G. BONI[1], F. NATALE[1,2], S. FUSCO[1,2], R. PIACENTINI[1,2], G. DE CHIARA[3], C. GRASSI[1,2];
[1]Neurosci., Univ. Cattolica del Sacro Cuore, Rome, Italy; [2]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [3]Inst. of Translational Pharmacol., Natl. Res. Council, Rome, Italy

Abstract: Neuroinflammation and oxidative stress play central roles in the onset and progression of Alzheimer's disease (AD). Microglial activation and abnormal production of reactive oxygen species (ROS) are among the earliest pathological changes identified as key contributors to synaptic dysfunction and cognitive impairment. To investigate the interplay among oxidative stress, inflammation, and microglial activation during the early stages of AD pathogenesis, we utilized two preclinical models, i.e., the triple-transgenic (3×Tg-AD) mouse model, representing early-onset familial AD, and the Herpes Simplex virus type 1 (HSV-1) mouse model, which mirrors sporadic AD. In both models we detected a sustained oxidative imbalance, characterized by ROS overproduction and a compensatory upregulation of antioxidant enzymes, including heme oxygenase-1 (HO-1) and superoxide dismutase 1 (SOD1). These changes were accompanied by elevated levels of 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation, and a significant upregulation of the NLRP3 inflammasome, which is a major regulator of innate immune responses. Importantly, both 3×Tg-AD and HSV-1 mice displayed consistent increases in key inflammatory mediators, including Interleukin-1β, Interleukin-2, and RANTES. These cytokines and chemokines promote neuroinflammation and modulate microglial behavior, thus triggering chronic immune activation in the central nervous system. Detailed morphological and molecular analyses of microglia revealed a shift toward an activated phenotype, evidenced by elevated expression of CD68, increased soma size, and reduced branching complexity—hallmarks of proinflammatory microglial activation. These neuroimmune alterations were accompanied by significant deficits in long-term potentiation at CA3-CA1 hippocampal synapses and hippocampus-dependent memory, as assessed by the Novel Object Recognition and Object Place Recognition tasks. Collectively, our findings identify oxidative stress and

neuroinflammation as convergent and early pathogenic mechanisms in AD, underscoring their relevance as potential targets for disease-modifying therapeutic strategies.

Disclosures: R. Sollazzo: None. **D.D. Li Puma:** None. **M. Rinaudo:** None. **F. Paciello:** None. **S. Autelitano:** None. **G. Aceto:** None. **G. Boni:** None. **F. Natale:** None. **S. Fusco:** None. **R. Piacentini:** None. **G. De Chiara:** None. **C. Grassi:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.03/E12

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

Support: Alzheimer's Association 24AARF-1241864
Edith M. Laudati Fund

Title: Early adult microglial and global C1q deletion yield divergent effects on amyloid pathology and synaptic engulfment in an Alzheimer's Disease mouse model

Authors: *T. J. PETRISKO, S.-H. CHU, B. ZHANG, A. J. TENNER;
UC Irvine, Irvine, CA

Abstract: The complement system, while having protective roles within the CNS, also contributes to enhanced inflammation and cognitive decline in Alzheimer's disease (AD). Previous reports showed that constitutive genetic deletion of C1q reduces glial activity and attenuates neuronal loss in AD mouse models. It is now known that microglia are the primary producers of C1q within the CNS. Therefore the aggressive Arctic (Arc) AD mouse model was used to determine if early adult microglial and/or global deletion of C1q would reduce engulfment of synapses, glial reactivity and amyloid plaque pathology.Briefly, C1qa$^{FL/FL}$ and Arc C1qa$^{FL/FL}$ mice with and without the RosaCre^{ERT2} transgene were treated with tamoxifen at 11-wks of age to induce global C1q deletion (designated iC1qKO). C1qa$^{FL/FL}$CX3CR1Cre^{ERET2} (designated C1q$^{\Delta MG}$) and ArcC1q$^{\Delta MG}$ mice, previously shown to delete microglial C1q by 8 weeks of age in the absence of tamoxifen, were also generated. At 10m of age, hippocampi were collected and confocal and immunofluorescence microscopy (IF) was used to quantify microglial engulfment of the synaptic marker Vglut1, lysosome-associated engulfment of amyloid by glia, glial activation (Iba1/GFAP), C3 and C5aR1 expression, as well as amyloid pathology. Engulfment of Vglut1 within microglial lysosomes was significantly reduced in hippocampal CA1 of ArcC1q$^{\Delta MG}$ mice and in both the CA1 and CA3 subregions in Arc^{iC1qKO} mice compared to C1q-sufficient Arc controls. Expression of C3 was significantly reduced in both ArcC1q$^{\Delta MG}$ (37%) and Arc^{iC1qKO} mice (48%), although no changes in C5aR1 expression nor astrocyte (GFAP) or microglia (Iba1) reactivity was observed. Early adult global deletion of C1q significantly reduced amyloid plaque burden levels by both IF (33%) and MSD (41%) assay for insoluble amyloid but did not alter lysosomal associated amyloid. In contrast, microglial deletion

of C1q failed to reduce amyloid levels despite reducing the amount of CD68 colocalized with amyloid, within both microglial (48%) and astrocytes (43%). These findings support the role of C1q in synapse engulfment and astrocytic C3 induction . The data are also consistent with the hypothesis that peripheral C1q can contribute to amyloid plaque load, either directly or by modulating peripheral inflammation.

Disclosures: T.J. Petrisko: None. **S. Chu:** None. **B. Zhang:** None. **A.J. Tenner:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.04/E13

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

Support: NIH Grant 1RF1AG074256-01A1

Title: Extracellular Vesicles from Human iPSC-derived Astrocytes Can Restrain Neuroinflammation and Maintain Better Brain Function in a Model of Early Onset Alzheimer's Disease

Authors: *A. K. SHETTY, L. N. MADHU, S. RAO, Y. SOMAYAJI, S. VENKATA GANESH, M. KODALI, S. KOTIAN, B. SHUAI, G. SHANKAR, S. ATTALURI, V. RAO; Cell Biol. and Genet., Inst. for Regenerative Medicine, Texas A&M Univ. Col. of Med., College Station, TX

Abstract: Alzheimer's disease (AD) is a progressive disease where dementia symptoms worsen over several years. Neuroinflammation plays a crucial role in the development and progression of AD. Extracellular vesicles (EVs) shed by human-induced pluripotent stem cell (hiPSC)-derived astrocytes (hA-EVs) could be a promising therapeutic approach for neuroinflammation in AD, as they carry antiinflammatory molecules. This study examined the efficacy of multiple intranasal (IN) administrations of hA-EVs, purified through chromatographic methods from cultures of hiPSC-derived astrocytes, for reducing neuroinflammation and maintaining better cognitive function for extended periods in 5xFAD mice. Three-month-old 5xFAD female mice received IN administrations of hA-EVs (30 x 109 EVs) or the vehicle (once monthly for 5 months). A month after the final dose of EVs (i.e., at 8 months of age), cognitive and mood functions were assessed through a series of neurobehavioral tests, and animals were euthanized for brain tissue harvesting and quantification of markers of neuroinflammation and amyloid plaques. 5xFAD mice receiving monthly treatment to hA-EVs displayed improved proficiency to discern minor changes in the environment in an object location test, object recognition memory in a novel object recognition test, and associative recognition memory in an object in place test, compared to 5xFAD mice receiving vehicle treatment. Besides, hA-EVs-treated 5xFAD mice displayed no anhedonia in a sucrose preference test compared to significant anhedonia in 5xFAD mice receiving vehicle treatment. Brain tissue analyses revealed that 5xFAD mice receiving hA-

EVs exhibited reduced microglial clusters, astrocyte hypertrophy, and NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome complexes within microglia in the hippocampus, compared to vehicle-treated 5xFAD mice. 5xFAD mice receiving hA-EVs also displayed significant reductions in concentrations of mediators (NLRP3, ASC, and cleaved caspase 1) and end products (interleukin-1 beta [IL-1β] and IL-18) of NLRP3 inflammasome activation. Additionally, the concentrations of proteins linked to the activation of cyclic GMP-AMP synthase and stimulator of interferon genes (cGAS-STING) signaling, such as phosphorylated STING and interferon-alpha, were reduced. The results suggest that intermittent intranasal administrations of hA-EVs can maintain better cognitive and mood function for prolonged periods in an early-onset model of AD by significantly restraining the progression of neuroinflammation.

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Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.05/E14

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

Title: Assessing humanized APOE mouse models: A comparative study for neurodegenerative disease applications

Authors: *N. WANG[1], G. YU[3], Z. WANG[4], A. ALNOBANI[1], F. QI[2], M. C. WREN[1], M. WANG[3], B. ZHANG[3], J. PENG[4], L.-J. WU[2], T. KANEKIYO[1], C.-C. LIU[1], G. BU[1]; [1]Mayo Clin., Jacksonville, FL; [2]Mayo Clin., Rochester, MN; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Structural Biol., St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Humanized *APOE* targeted replacement (TR) mice are foundational tools for studying apoE biology in Alzheimer's disease (AD) and related dementias. Despite their widespread use, existing *APOE* mouse models, generated with different gene targeting strategies, have not been directly compared in terms of apoE isoform expression, lipid profiles, or transcriptomic signatures. Such differences could significantly impact how we interpret APOE and disease phenotypes, as well as underlying molecular mechanisms. In this study, we conducted a comprehensive molecular comparison of three humanized *APOE* mouse lines, all on a C57BL/6 background, sourced from Taconic, the Cure Alzheimer's Fund, and The Jackson Laboratory. We assessed apoE protein and transcript levels, peripheral plasma lipid composition, and bulk brain transcriptomics. Through biochemical and proteomic measurements, we found that apoE isoforms exhibited differential expression levels among the three sources, in brain, liver, and plasma. Peripheral lipids including LDL, HDL, cholesterol, and triglycerides also showed distinct concentrations in each source/genotype. Through Weighted Gene Co-expression

Network Analysis (WGCNA), we determined that "source" had the greatest impact on brain transcriptomes. More importantly, we identified distinct brain transcriptional signatures among these mouse models, which were influenced by source, *APOE* genotype, and sex. Finally, we analyzed differentially expressed genes (DEGs) among these models based on source, genotype, and sex differences. Our findings highlight *APOE* genotype- and source-dependent variations in apoE isoform expression, lipid profiles, and key regulatory pathways, emphasizing the need for careful selection of appropriate lines to align with specific experimental objectives. These results underscore the importance of consistency and caution in choosing and utilizing humanized *APOE* mouse models, offering molecular insights into key apoE-related outcomes relevant to aging, AD, and other apoE-associated conditions. Keyword: APOE, Alzheimer's disease, mouse model, lipid, transcriptomics

Disclosures: N. Wang: None. **G. Yu:** None. **Z. Wang:** None. **A. Alnobani:** None. **F. Qi:** None. **M.C. Wren:** None. **M. Wang:** None. **B. Zhang:** None. **J. Peng:** None. **L. Wu:** None. **T. Kanekiyo:** None. **C. Liu:** None. **G. Bu:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.06/E15

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

Support: NIA Grant U54 AG054345
NIA Grant U54 AG054349

Title: Bridging the gap between mouse and human APOE with a next-generation model of Alzheimer's disease

Authors: *N. E. KWANG, C. A. PREKOPA, G. MILINKEVICIUTE, K. N. GREEN, V. SWARUP;
Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Mice are the most extensively used model organism in Alzheimer's disease (AD) research, yet they frequently fail to reproduce essential features of human AD pathology. APOE, the greatest genetic determinant of AD risk, differs significantly between mouse and human, sharing only 70% sequence homology and exhibiting differences in protein structure and lipid binding ability. This translational gap highlights the need to characterize the differences in how mouse and human APOE influence cellular responses to pathology. The MODEL-AD consortium recently developed a next-generation mouse model, MODEL-AD2, which features replacements of mouse Aβ, *Mapt*, and *Apoe* with their humanized counterparts - human $A\beta^{SWE/IB\text{-}loxP}$, *MAPT*, and *APOE4*. To understand the impact of inserting human *APOE4* (*hAPOE4*) into the mouse genome, we compared 12-month-old MODEL-AD2 mice ($hA\beta^{SWE/IB\text{-}loxP}$; *hMAPT*; *hAPOE4*) to mouse *Apoe* (*mApoe*) controls ($hA\beta^{SWE/IB\text{-}loxP}$; hMAPT) and wildtype

controls using immunofluorescence (IF; n=5/sex/genotype) and single-cell spatial transcriptomics (scST; n=3/sex/genotype) using the Nanostring CosMx. MODEL-AD2 mice carrying *hAPOE4* had fewer plaques in the cortex relative to *mApoe* controls, yet had increased cerebral amyloid angiopathy (CAA), a commonly co-occurring vascular pathology in human AD where Aβ deposits form in the blood vessels of the brain. This finding suggests that relative to mApoe, hAPOE4 shifts amyloid deposition from the parenchyma to the vasculature, recapitulating a key feature of human AD pathology. At the transcriptome level, MODEL-AD2 mice showed significant gene expression changes across the brain, but most notably in the choroid plexus (a brain region with high *APOE* expression), compared to *mApoe* mice. Additionally, along with the reduction in plaque load, we observed a reduction in the total number of disease-associated microglia surrounding plaques in MODEL-AD2 mice relative to *mApoe* controls. This work highlights the functional differences between mouse and human *APOE*, underscoring the nuance required in interpreting results from mouse studies. Our findings suggest that humanization of *APOE* in mouse models may better recapitulate aspects of human AD pathology, particularly in terms of amyloid distribution and cellular responses.

Disclosures: N.E. Kwang: None. **C.A. Prekopa:** None. **G. Milinkeviciute:** None. **K.N. Green:** None. **V. Swarup:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.07/E16

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

Support: NIH Grant 3RF1AG061001-01S1
NIH Grant P51OD011107-59S6

Title: Sars-cov-2 infection and spreading is facilitated by beta-amyloid presence in the aging brain

Authors: *D. BECKMAN, G. B. DINIZ, S. OTT, K. VAN ROMPAY, J. MORRISON; Univ. of California, Davis, CA

Abstract: The pandemic caused by COVID-19 has resulted in a significant public health crisis, with over 775 million cases and more than 7 million deaths worldwide. Recent reports reveal that over 80% of patients experience neurological symptoms, ranging from loss of smell and brain fog to severe conditions like hemorrhagic stroke and development of psychiatric disorders. Older individuals have been particularly vulnerable to acute and chronic SARS-CoV-2 infection, particularly in the presence of comorbidities such as Type 2 diabetes (T2D) and hypertension. We recently described that in rhesus monkeys, SARS-CoV-2 is found in olfactory brain areas at 7 days post infection, with neurons being the primary target of productive infection, and the pathology is worsened by aging and diabetes in infected monkeys. Taking advantage that rhesus

macaques normally develop amyloid plaques with aging, we sought to investigate whether or not SARS-CoV-2 presence in the brain is increased in regions displaying amyloid accumulation and neuroinflammation, potentially accelerating age-related neurodegeneration. Our analysis compared the brains of four SARS-CoV-2 infected rhesus macaques with T2D to those of eight age-matched controls, both with and without T2D. Using quantitative high-resolution microscopy, our analysis shows that SARS-CoV-2 presence is enhanced in regions with amyloid plaques such as the entorhinal and orbitofrontal cortices as compared to young infected brains and non-amyloid regions of the infected aged brains. Furthermore, we observed a significant disruption of the blood-brain barrier, characterized by intense microglia activation and the recruitment of neutrophils in aged, infected monkeys compared to their non-infected age-matched controls. These findings indicate that COVID-19 infection may accelerate neuroinflammatory responses linked to β-amyloid pathology presence in aged non-human primates, highlighting the possibility of an increase in future incidence of Alzheimer's Disease (AD) following the COVID-19 pandemic. Considering the increasing social and economic costs associated with AD and the increasing age of our population, it is critical that we understand a possible synergistic effect between SARS-CoV-2 infection and major pathological events associated with aging and AD.

Disclosures: D. Beckman: None. **G.B. Diniz:** None. **S. Ott:** None. **K. Van Rompay:** None. **J. Morrison:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.08/E17

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

Title: Evaluating the therapeutic potential of GDNF-secreting neural progenitor cells in a mouse model of Alzheimer's disease

Authors: *V. MOSER[1], L. DIMAS-HARMS[2], M. ALCANTARA[2], P. AVALOS[2], C. N. SVENDSEN[2];
[2]Regenerative Med. Inst., [1]Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Glial-derived neurotrophic factor (GDNF) is a promising therapeutic protein, as it promotes survival of key neuronal populations, including dopamine neurons in Parkinson's disease, and motor neurons in amyotrophic lateral sclerosis (ALS). However, as GDNF does not cross the blood brain barrier, it requires direct delivery to the central nervous system. We have previously developed a line of human fetal-derived neural progenitor cells (NPCs), transduced to express GDNF, and demonstrated that NPC-GDNF cells are neuroprotective. Specifically, NPC-GDNF protect motor neurons and photoreceptors in animal models of ALS and retinitis pigmentosa, respectively, and our lab has advanced this cell therapy to Phase I/IIa clinical trials for these indications. We are now expanding on this work in order to test the therapeutic

potential of NPC-GDNF cells in the context of Alzheimer's disease (AD). NPC-GDNF cells were transplanted into 2 regions of the dentate gyrus of the 5xFAD mouse model of AD. Transplantation was performed at 3-months-of-age, when amyloid pathology and neuroinflammation are just beginning in this animal model. Learning and memory were assessed in the spontaneous alternation behavior task and in novel object location and recognition, and nesting behavior, which is often disrupted in 5xFAD mice, was evaluated. Animals were euthanized 4-6 months after transplantation, and graft survival and GDNF secretion were assessed immunohistochemically. Additional outcome measures include neural health and survival, as measured by VGLUT1 and NeuN, doublecortin and Ki-67 to evaluate neurogenesis, and microgliosis and astrogliosis as assessed by IBA1/CD68 and GFAP, respectively. Results are expected to demonstrate whether the therapeutic potential of NPC-GDNF cells extends to neurodegenerative conditions outside of ALS and retinitis pigmentosa, and will indicate their potential for clinical trials as a cell and gene therapy-based treatment for AD.

Disclosures: V. Moser: None. **L. Dimas-Harms:** None. **M. Alcantara:** None. **P. Avalos:** None. **C.N. Svendsen:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.09/E18

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

Support: R01AG072899
R01AA017729

Title: Interferon response factor 3 (IRF3) regulates neuroinflammation in acute inflammatory conditions of LPS challenge and in Alzheimer's disease.

Authors: *R. JOSHI;
Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA

Abstract: The pathophysiological role of interferon signaling is emerging in various neurological disorders. Interferon response factor 3 (IRF3) is one the key transcription factors leading to Type-I IFN production and is a regulator of immune signaling pathways (for example- cGAS-STING pathway, TLR4 and NLRP3 inflammasome signaling) implicated in neuroinflammatory diseases. Yet the specific role of IRF3 in neuroinflammatory responses of microglia remains poorly studied. We tested the role of IRF3 in two neuroinflammatory milieu- LPS challenge and Alzheimer's Disease (AD) using IRF3KO mouse model and microglia cultures. After acute (6h) LPS (1mg/kg) challenge IRF3KO mice showed significantly attenuated sickness behavior, cortical cytokine response (IL1β, IL1α, MCP-1, CxCL1), microglial reactivity (*isg56, gbp2, cox-2*, *H2-D1* mRNA) and astrocyte activation (*gfap, gbp2, igtp, isg56* mRNA) compared to the WT-LPS group. Also, deletion of IRF3 in microglia delayed TLR4-mediated

signaling in primary microglia cultures, verifying the direct role of IRF3 in microglia. Moreover, the mere expression of a constitutively active IRF3 (S388/390D -IRF32D) in microglia led to the upregulation of genes (*apoe, axl, itgax, ctsb*) characteristic of disease-associated microglia in AD. In addition, Aβ challenge to wild-type microglia cultures showed significant upregulation of IRF3 phosphorylation. These results prompted us to assess the role of IRF3 in an AD mouse model (5XFAD). RNA sequencing of microglia from IRF3KO-5XFAD mice showed a near-complete absence of Interferon response microglia (IRMs). Surprisingly, we observed increased Aβ pathology and worsened memory in IRF3KO-5XFAD mice compared to the WT-5XFAD. In addition, we observed reduction in phagocytosis and antigen presentation-related transcripts in microglia from IRF3KO-5XFAD, suggesting reduced clearance potential. Lastly, we identified Zbp1 as a mediator of inflammatory changes induced by IRF3 in models of LPS challenge and AD. Our results show that IRF3 is a critical component of LPS-induced pro-inflammatory responses and astrocyte activation mediated by microglia. In addition, IRF3 deletion exacerbates amyloid plaque pathology and alters microglia responses in AD suggesting a complex regulatory role for IRF3 in different neuroinflammatory context.

Disclosures: R. Joshi: None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.10/E19

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

Support: R01AG075998
R01AG056478
R01AG055865
AG056478-04S1
AARG-NTF-21-846586

Title: Identification of Chlamydia pneumoniae and associated NLRP3 inflammasome activation in Alzheimer’s disease retina

Authors: *B. GAIRE, Y. KORONYO, J.-P. S. VIT, A. HUTTON, A. RENTSENDORJ, D.-T. FUCHS, L. SUBEDI, N. SWERDLOW, K. L. BLACK, J. G. MEYER, M. ARDITI, T. R. CROTHER, M. KORONYO-HAMAOUI;
Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Motivation: Recent advances in Alzheimer’s disease (AD) research highlight the retina as an emerging CNS target for noninvasive AD diagnosis and monitoring, with parallels between retinal and brain AD biomarkers. While the etiology of AD pathogenesis remains unclear, an emerging theory links AD to microbial infection as a plausible contributor to disease progression. Indeed, the presence of several microbes, including *Chlamydia pneumoniae* (Cp),

has been reported in the post-mortem brains of AD patients, but its role in the AD retina has not been studied. Here, we investigate the presence and distribution of Cp infection and its associated inflammasome activation in the AD retina. **Approach**: We used a range of histological, biochemical, and machine learning techniques to detect Cp and associated biomarkers in the postmortem retina and brain of patients with mild cognitive impairment (MCI due to AD) and AD dementia, compared to retinas from age- and sex-matched individuals with normal cognition (NC). We assessed Cp presence and its potential relationship with NLRP3 inflammasome activation, gliosis, and pyroptosis/apoptosis. **Results**: We identified Cp inclusions in the retina and brain tissues of MCI and AD patients, with a 3- to 4-fold higher burden in AD, but not in MCI, compared to NC controls, suggesting Cp infection expands in later AD-dementia stages. Mass spectrometry analysis of the brain and retinal proteomes revealed similar gram-negative bacterial infection-related proteins, dysregulated *Chlamydia*-interactome proteins, and common inflammatory and cellular degeneration pathways in the brains and retinas of AD patients. Retinal Cp was closely associated with increased retinal $A\beta_{42}$ and activation of NLRP3 inflammasome components, NLRP3, caspase-1, and apoptosis-associated spec-like protein (ASC) in AD, triggering cleaved caspase-3 apoptotic and $GSDMD^{Nterm}$ pyroptotic cell death. Retinal microglia were directly involved in phagocytosis of Cp-infected cells, though the relative Cp-associated microglial population was substantially reduced in AD. Higher retinal Cp burden was observed in APOE ε4 carriers and subjects with more severe brain pathology (neurofibrillary tangles, Braak stage, and cognitive dysfunction). Machine learning models revealed that retinal Cp or NLRP3, combined with retinal $A\beta_{42}$, may effectively predict AD diagnosis, Braak stage, and cognition. **Conclusions**: This study identifies increased Cp burden in the retinas and brains of AD patients associated with early inflammasome activation linking neurodegeneration, proposing new therapeutic avenues involving antibiotic and inflammasome-attenuation treatments.

Disclosures: B. Gaire: None. **Y. Koronyo:** None. **J.S. Vit:** None. **A. Hutton:** None. **A. Rentsendorj:** None. **D. Fuchs:** None. **L. Subedi:** None. **N. Swerdlow:** None. **K.L. Black:** None. **J.G. Meyer:** None. **M. Arditi:** None. **T.R. Crother:** None. **M. Koronyo-Hamaoui:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.11/E20

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

Support: JPND Research Grant JPND2022-148
Bethlehem Griffiths Research Foundation Grant BGRF2403

Title: Microglia mediate the cognitive benefits of environmental enrichment in wild-type mice and the 5xFAD transgenic mouse model of Alzheimer’s disease

Authors: *H. LIAO[1,3], S. TSANG[4], A. ZHANG[4], D. LU[4], B. KAMINSKA[5], R. PAOLICELLI[6], B. J. TURNER[2], A. J. HANNAN[3];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive decline, memory loss, and behavioral changes. Microglia, the brain's main immune cells, contribute to AD pathology when dysregulated. Environmental enrichment (EE), which provides cognitive, sensory and motor stimulation, has shown potential in delaying disease onset and mitigating AD progression. This study explores microglia's role in mediating EE effects in wild-type (WT) and 5xFAD mice, a transgenic model of AD. The results demonstrated that PLX3397 treatment (290 mg/kg, 4-32 weeks of age) partially depleted microglia, with greater depletion in WT than in 5xFAD mice. Behavioral tests conducted between 24 and 32 weeks of age revealed that in the 5xFAD mice, males exhibited reduced body weight, while both sexes showed enhanced motor coordination and balance, reduced anxiety, and decreased exploratory behavior. EE from 9 to 32 weeks reduced anxiety in WT mice. Additionally, EE improved spatial learning and memory in 5xFAD mice, with a greater effect observed when compared to enriched WT mice. EE also boosted locomotor function, motor coordination, balance, novelty exploration, and brain weight across both genotypes. However, these EE-induced improvements in spatial learning and memory in both genotypes were abolished in the PLX3397-treated group, suggesting that microglia might play a critical role in mediating these cognitive benefits. Despite partial microglia depletion, genotypic and housing effects on motor function, anxiety, and body/brain weight remained unchanged. These findings demonstrate an essential role for microglia in mediating EE-induced cognitive benefits in AD, providing further evidence that targeting microglial function could be a potential therapeutic approach for improving cognitive outcomes in neurodegenerative diseases such as AD and other forms of dementia.

Disclosures: H. Liao: None. **S. Tsang:** None. **A. Zhang:** None. **D. Lu:** None. **B. Kaminska:** None. **R. Paolicelli:** None. **B.J. Turner:** None. **A.J. Hannan:** None.

Poster

PSTR381: Neuroimmune Mechanisms of Alzheimer's Disease I

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR381.12/E21

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

Support: Traget ALS

Title: Crosstalk between TDP43 and dsRNA

Authors: *P. SINHA[1], M. W. ALBERS[2];
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Abstract: TAR DNA-binding protein 43 (TDP43) is a key regulator of RNA metabolism whose cytoplasmic mislocalization and aggregation are central features of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). Double-stranded RNA (dsRNA), typically associated with viral defense pathways, has recently emerged as an endogenous trigger of innate immune activation in neurodegeneration. Emerging evidence suggests a previously unrecognized interplay between TDP43 and dsRNA, highlighting a novel axis of pathophysiological relevance that may contribute to disease progression.
In mouse models with dsRNA, we observe mislocalization of TDP43, a hallmark of ALS and AD pathology. Conversely, in TDP43 transgenic models, we detect the presence of endogenous cytoplasmic dsRNA. This bidirectional relationship suggests a potential cross-talk between TDP43 dysfunction and the accumulation of dsRNA. Importantly, our preliminary data indicate a direct interplay between TDP43 and dsRNA molecules, further supporting a mechanistic link. TDP43, known to regulate RNA metabolism and stress granule dynamics, may contribute to or be affected by dsRNA accumulation, leading to cytoplasmic mislocalization, aggregation, and neurotoxicity. Given the central role of TDP43 in multiple neurodegenerative diseases and the emerging contribution of innate immune triggers like dsRNA, elucidating this interaction could provide critical insights into disease progression. Understanding how TDP43 and dsRNA influence each other in vivo may open new avenues for therapeutic intervention targeting their pathological loop.

Disclosures: P. Sinha: None. **M.W. Albers:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.01/E22

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

Title: Amyloid-beta Positron Emission Tomography for predicting time to conversion of mild cognitive impairment to Alzheimer's disease

Authors: *S. CHEN, R. J. ELLIS;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects more than 55 million people worldwide. Identifying individuals with mild cognitive impairment (MCI) who are most likely to progress to AD is key for early preventative treatment. Previous models are limited by single-cohort training, inadequate consideration of subgroup differences, and reliance on static, binary classification methods. To address these gaps, this study aims to

conduct a multi-cohort survival analysis to predict temporal progression of MCI to AD. Amyloid-beta positron emission tomography (PET) imaging, clinical and genotype data from the ADNI, AIBL, NACC, and OASIS databases were used to build a Cox proportional hazards model with time-varying covariates, with Alzheimer's diagnosis as the outcome. We implemented cross-cohort validation, where the model was trained on three of four cohorts and then validated on a held-out fourth cohort, providing a more robust estimate of the model's generalizability across diverse patient groups. The fitted model was used to calculate risk scores for each patient within the training and test set to assess generalizability between datasets. Given that clinical decision-making requires a discrete prediction, the threshold maximizing Youden's J statistic was applied to output a binary prediction of whether a person will convert or not. Each cohort included 400-500 MCI patients, with conversion rates to AD dementia ranging from 25-40% at last follow-up. A preliminary Cox proportional hazards model incorporating amyloid-beta PET imaging, demographics, and APOE genotype from ADNI and NACC has been fitted and used to generate individual-level risk scores. Early findings suggest that models incorporating amyloid-beta PET data and time-varying covariates demonstrated improved time-varying AUROC and concordance indices (C-index) compared to baseline models trained only on demographics and APOE genotype. Ongoing work will expand this approach to additional cohorts. Further, exploratory subgroup analysis suggested variation in model performance by education, sex, and APOE genotype, which we plan to investigate further. These early results demonstrate the feasibility of a comprehensive, survival-based prediction model for MCI to AD conversion using multiple cohorts with time-varying covariates. Next steps include the inclusion of additional cohorts and subgroup analyses stratified by education, sex, and APOE genotype to evaluate model performance across diverse patient profiles. The overall goal is to enable clinical integration of the model into a preventative treatment approach for AD patients.

Disclosures: S. Chen: None. **R.J. Ellis:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.02/E23

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

Title: Longitudinal dual-task study of gait changes in stable MCI patients

Authors: O. M. BAKKE[1], D. NEUHAUS[2], *C. RODRÍGUEZ-ARANDA[3];
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Abstract: Background: Gait deteriorates with increasing age and these alterations are more prevalent during dual-tasking in older individuals with mild cognitive impairment (MCI). To date, there is still scarce knowledge about the longitudinal progression of gait difficulties in this population associated with walking while performing a secondary task. Due to the heterogeneity

of the MCI condition, our study focuses on the assessment of MCI patients who have remained stable over time. **Aim**: Investigate changes in spatio-temporal gait parameters during single and dual-task conditions in stable MCI patients and healthy age-matched controls after an average period of 4.5 years from initial evaluation. **Method:** MCI patients (n = 16, *M* age = 68.81 yrs.) and healthy elders (n= 16, *M* age = 68.13 yrs.) participated in the study. Spatio-temporal gait parameters were acquired with the Optogait-system. The mean and coefficient of variation (CoV) were calculated for gait speed, step length and step width measurements during only walking (baseline) and in dual-tasking. Dual-tasking required subjects to walk and perform a dichotic listening (DL) task under 3 conditions: spontaneous attention, attending to right-ear, attending to left-ear. A series of factorial ANOVAs with repeated measures were conducted. **Results:** All gait parameters showed deterioration particularly in controls after 4.5 years of initial testing. Significant group differences existed across sessions. Healthy controls showed a reduction in Mean of gait speed across conditions on retest session, particularly when attending to right ear (MCI Test1 & Test 2 = .67 m/sec; Controls Test1 = 1.07 m/sec, Test2 = .76 m/sec). In turn, MCI patients at retest showed significant increments in step width CoV across conditions, most importantly during baseline (MCI Test1 = 99 %, Test 2 = 136.67 %; Controls Test1 = 98.02 %, Test2 = 87.88 %). Concerning performance in the DL task, a significant Group X Session interaction demonstrated that groups differed significantly on the retest session, in which MCI patients showed difficulties to direct attention to one specific ear. **Conclusions:** Despite group differences at test and retest sessions, MCI patients preserved mostly their gait characteristics longitudinally, while healthy controls developed a pattern of walking that resembles that of patients. However, a clear group differentiation existed at retest related to step width variability (CoV), which showed significant increments on the patient group in single and dual-task conditions. This finding points to unique progressive detrimental gait changes in cognitively stable MCI patients that may serve as a hallmark of the condition.

Disclosures: O.M. Bakke: None. **D. Neuhaus:** None. **C. Rodríguez-Aranda:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.03/E24

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

Title: Investigation of the neuroprotective role of extracellular vesicles from physical exercise and associated with FNDC5/irisin in preclinical models of Alzheimer's disease

Authors: *T. RODY[1], N. D. SILVA[2], T. HUNTER[2], A. DA CONCEICAO[4], J. MARINATTO[4], G. B. DE FREITAS[3], C. FERNANDES[6], V. BODART-SANTOS[7], M. V. LOURENCO[5], S. T. FERREIRA[8], F. DE FELICE[2];
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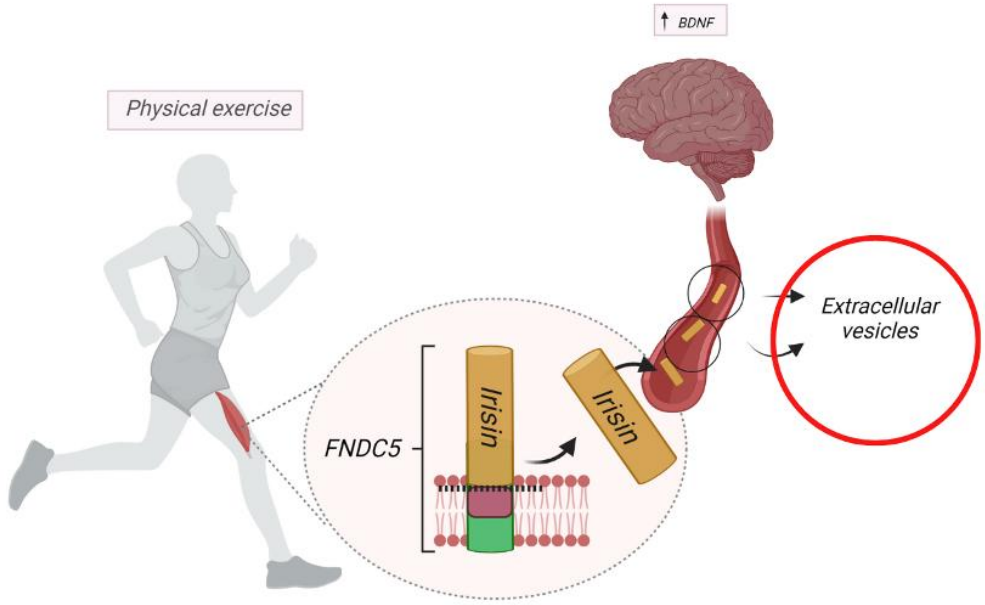
Janeiro, Rio de Janeiro, Brazil; [7]Dept. of Neurosci., Mayo Clin. Florida, Jacksonville, FL; [8]Fed. Univ. Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by neurodegeneration, neuroinflammation, and memory loss. Due to the lack of disease-modifying treatments, the search for new therapeutic strategies is necessary. Physical exercise has been investigated as a non-pharmacological therapy to prevent and improve AD, as preclinical studies demonstrate neuroprotective effects related to exercise in animal models, including improvements in learning and memory. Physical exercise upregulates irisin, a myokine derived from FNDC5, previously shown to mediate beneficial actions of exercise on memory in AD mouse models. Exercise also causes an overall increase in plasma extracellular vesicles (EVs). EVs are membranous particles that play an important role in communication between the periphery and brain, carrying biomolecules from donor to recipient cells, notably modulating levels of different myokines during exercise. Interestingly, EVs have been reported to modulate some physiological roles of FNDC5/irisin in peripheral tissues. However, the involvement of EV-associated FNDC5/irisin in the brain has not yet been investigated. Herein, we aim to investigate extracellular vesicles (EVs) as a potential therapeutic approach. We hypothesize that exercise modulates irisin production in EVs, which might elicit beneficial effects on cognition. Our results show that FNDC5/irisin is increased in plasma EVs of exercised mice and in mice that overexpress FNDC5. We report that intravenous treatment with EVs obtained from exercised wild-type mice, but not from exercised FNDC5 KO mice, elevates cortical BDNF expression and rescues memory in APP/PS1 mice, a transgenic AD model. In addition, EVs from mice overexpressing FNDC5/irisin also rescue memory in APP/PS1 mice. These results indicate that EVs may be necessary for FNDC5/irisin-related crosstalk between periphery and brain, providing insight into the role of EVs as carriers of FNDC5/irisin during exercise and highlighting the therapeutic potential of EVs carrying irisin to restore cognition in AD.



Disclosures: T. Rody: None. **N.D. Silva:** None. **T. Hunter:** None. **A. Da Conceicao:** None. **J. Marinatto:** None. **G.B. De Freitas:** None. **C. Fernandes:** None. **V. Bodart-Santos:** None. **M.V. Lourenco:** None. **S.T. Ferreira:** None. **F. De Felice:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.04/E25

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

Support: UIC Department of Chemistry Start-up Funds
NIH R35 GM152119
Cognition Therapeutics Fund

Title: Characterizing the ligand-sensitive TMEM97 interactome in Alzheimer's disease

Authors: *A. NGUYEN[1], Y.-S. CHENG[2], R. CHEN[3], A. ONDRUS[2];
[1]Dept. of Pharmaceut. Sci., [2]Dept. of Chem., [3]Univ. of Illinois, Chicago, Chicago, IL

Abstract: Loss of neuronal structure and function in neurodegenerative disease leads to disrupted neural circuitry and progressive cell death. In Alzheimer's disease (AD), binding and internalization of neurotoxic amyloid beta (Aβ) aggregates are significant drivers of neurodegeneration. Recent studies in Aβ pathology have identified transmembrane protein 97 (TMEM97), also known as the σ2 receptor, as a critical component of membrane receptor complexes that mediate neuronal Aβ internalization. TMEM97 is an established drug target for many neurological diseases, and TMEM97 ligands have recently completed successful Phase 2 clinical trials for curative AD treatment. However, the specific TMEM97 protein partners within these complexes, and the mechanisms by which TMEM97 ligands influence receptor function, are poorly understood. Our objective is to comprehensively profile the ligand-sensitive TMEM97 interactome and define the role of TMEM97 protein-protein interactions in AD pathology. Toward this objective, we have engineered a human embryonic 293T cell line lacking endogenous TMEM97 that stably expresses FLAG-tagged TMEM97 or control proteins ($n = 3$ / group). Using these cell lines, we have used co-immunoprecipitation/mass spectrometry (IP/MS) to capture proteome-wide TMEM97 interactors in the presence and absence of selective TMEM97 ligands. Our results demonstrate that TMEM97 engages extensively with AD-associated proteins, including the reported Aβ co-receptors PGRMC1 and LDLR. To identify global interaction networks relevant to AD and neurobiology, we prioritized our TMEM97 interactome analysis by cross-referencing to gene ontology (GO), Gene Set Enrichment Analyses (GSEA), and the Neuropro databases. GO analysis showed that TMEM97 protein networks are significantly ($p > 0.05$) associated with Aβ processing, neurodegeneration, mitochondrial respiration, and autophagy. Likewise, GSEA analysis identified that TMEM97 protein partners are enriched in KEGG pathways such as 'Alzheimer's disease' and 'Parkinson's disease'. We also established that TMEM97 ligands dose-dependently alter key interactions within these AD-

associated pathways. Overall, our work provides insight into TMEM97 pharmacology in the context of AD, paving the way to new treatment modalities for AD and other neurodegenerative pathologies. Our next steps are focused on investigating TMEM97 protein interactions and ligand phenotypes in neurons from wild-type and 5xFAD mice.

Disclosures: A. Nguyen: 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.; Cognition Therapeutics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cognition Therapeutics. **Y. Cheng:** None. **R. Chen:** None. **A. Ondrus:** 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.; Cognition Therapeutics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cognition Therapeutics.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.05/E26

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

Support: R01 CA172546
U01 AG032969
P01 CA186866
R56 AG061869
R01 AG067598
R01 AG074004
R01 AG072599

Title: Epichaperome Remodeling Defines Layer- and Cell-Type-Specific Vulnerability in the Prefrontal Cortex of Alzheimer's Disease Brains

Authors: *S. BAY[1], A. RODINA[1], F. HAUT[3], T. ROYCHOWDHURY[1], A. STANISZEWSKI[4], S. SHARMA[1], S. CHAKRABARTY[2], C. DIGWAL[1], A. STANISAVLJEVIC[6], A. LABUZA[9], M. J. ALLDRED[7], P. PANCHAL[1], E. ROSIEK[1], H. SASAGURI[10], T. C. SAIDO[11], J. SCHNEIDER[12], D. A. BENNETT[13], P. E. FRASER[14], H. ERDJUMENT-BROMAGE[15], T. A. NEUBERT[16], S. D. GINSBERG[8], O. ARANCIO[5], G. CHIOSIS[1];
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Abstract: Alzheimer's disease (AD) is marked by selective vulnerability of specific neuronal populations and cortical layers, yet the molecular drivers of this pattern remain unclear. Epichaperomes—stable, multimeric assemblies of chaperones and co-chaperones—have emerged as early markers of proteome dysfunction in AD, capable of rewiring protein interaction networks without requiring changes in gene expression (Inda et al. *Nature Communications* 2020, DOI: 10.1038/s41467-019-14082-5; Bay et al. *Res Sq* [Preprint]. 2025 Feb 12:rs.3.rs-5930673. doi: 10.21203/rs.3.rs-5930673/v1). These scaffolding assemblies form in vulnerable neurons and astrocytes and drive systems-level dysfunction across synaptic, metabolic, and inflammatory pathways. Notably, epichaperome disruptors such as PU-AD (Icapamespib) have entered clinical trials in AD, highlighting the translational potential of targeting epichaperome-based remodeling.Here, we used multiplex immunofluorescence staining and a chemical biology-based click probe to map the spatial and cell-type-specific distribution of epichaperomes in the dorsolateral prefrontal cortex (BA9) across the AD continuum. Using multiplex immunofluorescence staining of postmortem human BA9 tissue spanning non-cognitively impaired (NCI), mild cognitive impairment (MCI) and AD, we visualized epichaperome assemblies alongside neuronal (MAP2), astrocytic (GFAP), and nuclear (Hoechst) markers. We found that epichaperomes are largely absent in NCI brains but become prominent in MCI and AD, with distinct cell and layer-specific patterns. Earliest epichaperomes were observed in large pyramidal neurons of layers V and III and their neighboring astrocytes *prior* to overt structural abnormalities, suggesting that their appearance marks a molecularly vulnerable but potentially reversible cellular state.Our findings help establish a high-resolution anatomical map of early epichaperome remodeling in the human brain and implicate these pathologic assemblies as key regulators of region- and cell-specific dysfunction in AD. Our findings suggest that epichaperomes contribute to the molecular architecture of selective dysfunction in AD and provide tools to visualize and track this pathological remodeling in situ.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.06/E27

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

Support: NSFC Grant No. 82372081
NSFC Grant No. 82230041
NSFC Grant No. 91949205

Title: A mass-producible macaque model displays a durable Alzheimer-like cognitive deficit and hallmark amyloid-β, tau, neurofilament-light-chain pathologies.

Authors: *F. HE;
Res. Institute, Hubei Topgene Biotech., Wuhan, China

Abstract: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder characterized by cognitive deficit and pathological accumulation of amyloid-β (Aβ) and tau proteins. The rodent models have contributed greatly to unravel AD pathogenesis, but these AD models have been shown a modest clinical translational effectiveness. Therefore, developing mass-producible primate AD models is promising for more effective drug development. Here, we constructed the AD monkey models by simultaneously infusing AAV-Tau and Aβ into different brain regions. The induced monkeys showed a durable cognitive impairment lasting for at least 10 months after the modeling. Simultaneously, the increased levels of total tau and hyperphosphorylated tau (pTau) at several AD-associated sites, and neurofilament light chains (NfL) with altered Aβ level were detected at different time points in cerebrospinal fluid and/or plasma by using MSD kits. The increased brain accumulation of Aβ and tau proteins was also detected by positron emission tomography/magnetic resonance imaging and immunohistochemical staining. The model monkeys also had significant glial activation; an indicator of inflammation commonly seen in the brains of AD patients. Together, this study provides mass-producible monkey models showing durable AD-like hallmark pathologies (Aβ, tau, NfL, i.e., ATN) and cognitive deficits. As monkeys are genetically and metabolically the closest to humans, these models will offer more effective drug discovery and development for AD.

Disclosures: F. He: None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.07/E28

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

Support: USA Pennsylvania State Health Department grant Project 10: 420491-04400-02 to Nune Darbinian
NIH grant to Dr. Shohreh Amini
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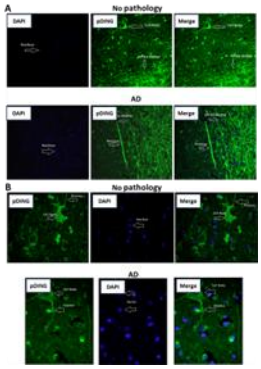
Dr. Schein (MD) Neuropathology Endowed Fellowship award to Dr. Darbinyan Armine, MD

Title: Neuroprotective Role of pDING Protein in the Aging Brain of Alzheimer's Disease (AD)Patients

Authors: *N. DARBINIAN[1,3], A. DARBINYAN[4], S. AMINI[2], M. E. SELZER[3];
[2]Dept. of Biology, Col. of Sci. and Technol., [1]Temple Univ., Philadelphia, PA; [3]Neural Sci., Lewis Katz Sch. of Med. At Temple Univ., Philadelphia, PA; [4]Dept. of Pathology, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Introduction: Previously, we provided evidence that DING phosphatase extracted from St. John's wort is neuroprotective against EtOH-mediated toxicity in rat and human fetal neurons *in vitro*. Now, we assess the role of the DING protein in the ageing brain and in Alzheimer's disease (AD). pDING (p38SJ), a member of the DING family of proteins, has been shown to be neuroprotective against cellular stress injury induced by alcohol, by HIV-1, and in cancer cells. p38SJ/DING protein has demonstrated phosphatase activity on MAPK substrates, but the effect of pDING on Tau phosphorylation, which is involved in AD, has not yet been explored. **Methods:** Expression of DING protein levels was studied in human postmortem brain by histochemistry and by quantitative western blot assay. Statistical testing included ANOVA. **Results:** Increased pDING, when transduced and overexpressed, was associated with increased cell survival. In aged brains (age =72-92 years), endogenously expressed 38 kDa pDING levels were associated with Tau dephosphorylation. Here, pDING is shown to be present in both normal and Alzheimer's disease-affected human brain tissue in neuronal cell bodies and processes. pDING demonstrated phosphatase activity in PC12 cells (a cell line derived from rat pheochromocytoma) and inhibited Tau phosphorylation in human brain tissue (both normal and AD) and in PC12 cells. **Conclusions**: Excess Tau phosphorylation leads to destabilization of microtubules and the formation of neurofibrillary tangles in neurons, a hallmark of neurodegeneration in Alzheimer's disease. pDING reduces the viability of proliferating cells compared to cells overexpressing Tau, while increasing cell viability in non-proliferating neuronal cells. This data suggests that pDING inhibits the phosphorylation of Tau and may have a potential role in AD. **pDING protein (green) is expressed in neurons in aging and AD brain tissues**.



Disclosures: N. Darbinian: None. **A. Darbinyan:** None. **S. Amini:** None. **M.E. Selzer:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.08/E29

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

Support: NIH R01 AG066080
NIH R01 AG071656
Alzheimer's Association PTC-17-439955

Title: Long-term persistence and safety of AAV-NGF gene therapy beyond 10 years in human Alzheimer's disease

Authors: *H. ZIA[1], Y. MAGAÑA[1], D. BARBA[2], B. T. HARRIS[3], V. GOODWILL[1], M. H. TUSZYNSKI[1,4], M. J. CASTLE[1];
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Abstract: Gene therapy is a rapidly evolving technology that is already approved for several human diseases, including spinal muscular atrophy. Adeno-associated virus (AAV) vectors are the most common vector for clinical gene delivery, but questions have arisen regarding their long-term safety and risk of inflammation. Here we report that expression of nerve growth factor (NGF) persists for more than 10 years without apparent toxicity after direct brain injection of AAV2-NGF in early Alzheimer's disease (AD).
In 2001, we initiated the first clinical program of gene therapy in early AD to test the hypothesis that the neurotrophic factor NGF would preserve cholinergic neuron function. Degeneration of cholinergic neurons occurs early in AD and contributes to cognitive decline. Preclinical studies found that NGF prevents cholinergic neuron death and stabilizes cholinergic projections to the cortex and hippocampus. Over the next 15 years, a total of 33 early AD patients received NGF gene delivery by direct injection of AAV2-NGF targeting the nucleus basalis of Meynert. In Phase 2 of the clinical trial, AAV2-NGF did not slow cognitive decline compared to sham surgery controls, but most injections (>90%) missed their target or failed to spread to cholinergic neurons as determined by pathological analysis of 10 cases.
We examined these 10 brains to assess the safety of AAV2-NGF injection, given recent reports that AAV2 may cause inflammation and cellular toxicity. We found no evidence of cellular toxicity or neuronal dropout by H&E stain or by immunolabeling for markers of T and B lymphocytes, microglia, or astrocytes. While microglia were present in regions of NGF expression, they were not associated with neuronal loss. Moreover, NGF gene expression remained detectable for more than 10 years after treatment.
We conclude that AAV2 supports safe and long-lasting gene expression beyond 10 years in the degenerating human brain.

Disclosures: H. Zia: None. **Y. Magaña:** None. **D. Barba:** None. **B.T. Harris:** None. **V. Goodwill:** None. **M.H. Tuszynski:** None. **M.J. Castle:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.09/E30

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

Support: NIH Grant 1R01AG066080-01A1

Title: Strong cortex-specific promoters for gene therapy in Alzheimer's disease

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Abstract: Gene therapy is a promising approach for cortical disorders such as Alzheimer's disease. Emerging adeno-associated virus (AAV) capsids can deliver genes across the blood-brain barrier and broadly treat the cerebral cortex, but improved promoters are needed to restrict gene expression to cortical neurons. For the greatest potential therapeutic efficacy, such promoters must drive strong gene expression as well as being small and specific. Here, we present several new cortex-specific promoters combining different enhancer sequences with the same human core promoter that potently restricts expression to forebrain neurons. The size of these promoters ranges from 2.5 kb to 1.1 kb.
Candidate promoters were first screened in mice by systemic delivery of AAV-PhP.eB carrying the eGFP reporter, and were compared against the common chicken beta-actin (CAG) and human synapsin 1 (hSyn1) promoters. Three candidates demonstrated significantly enhanced cortical eGFP expression compared to the CAG and hSyn1 promoters, with up to 3.0-fold greater expression than hSyn1 in the hippocampus and 1.4-fold greater in the entorhinal cortex by eGFP immunolabeling. These lead candidate promoters drove stronger cortical gene expression than hSyn1 despite being packaged as single-stranded AAV, while the hSyn1-eGFP control was packaged as self-complementary AAV. Off-target expression by these cortical promoters was reduced by up to 83% relative to hSyn1 in regions including cerebellum, brainstem, striatum, and thalamus. Expression was highly specific for cortical neurons, with no detectable expression in glial cells. These cortical promoters target both GABAergic inhibitory neurons and glutamatergic excitatory neurons labeled by RNAscope in situ hybridization. All tested cortical promoters maintained long-term gene expression without silencing: no change in eGFP fluorescence was observed more than 1 year after treatment. In a single 7-year-old male rhesus macaque monkey, these new promoters drove strong, neuron-specific gene expression after direct injection of AAV-PhP.eB into primate prefrontal cortex. We conclude that these cortex-specific promoters drive stronger gene expression in rodent and non-human primate cortical neurons than any previously described promoter sequence, with long-term persistence of cortical expression and greatly reduced off-target expression in other regions and cell types. These new promoters are

lead candidates for human gene therapy and could improve the safety and efficacy of therapeutic gene delivery in cortical disorders such as Alzheimer's disease.

Disclosures: B.I. Lu: None. **Y. Magaña:** None. **B. Chavez:** None. **K. Nakashima:** None. **A. Torreblanca-Zanca:** None. **P.A. Desai:** None. **R. Wurr:** None. **R. Macon:** None. **E. Sinopoulou:** None. **M.H. Tuszynski:** None. **M.J. Castle:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.10/E31

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

Support: Roneet Carmell Memorial Endowment Fund

Title: Non-invasive cervical vagus nerve stimulation enhances cerebral autonomic regulation and cognitive habituation in alzheimer's transgenic mice

Authors: *B. CHUNG[1], A. MOHTASEBZADA[1], S. BERDUO[1], K. SUAYNGAM[1], R. LOTFY[1], M. RAFI[1], S. KHATAMI[1], S. ANDALIB[4], Y. AKBARI[2,5,3];
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Abstract: Vagus Nerve Stimulation (VNS) is a technique that delivers electrical impulses to the vagus nerve, exerting neuromodulatory effects via autonomic and neuroimmune pathways. FDA has approved non-invasive VNS to treat migraines, epilepsy, and depression. Non-invasive transcutaneous auricular VNS (taVNS) modulates key brain regions such as the hippocampus, locus coeruleus, and nucleus tractus solitarius, making it a promising strategy for enhancing cognition in Alzheimer's Disease (AD). However, compared to taVNS, the effects of non-invasive cervical VNS (cVNS) on the autonomic nervous system and cognition in AD remain unclear. We hypothesized that cVNS may exert behavioral effects by impacting cerebral autonomic regulation. To address this, male and female transgenic 5x Familial Alzheimer Disease (FAD) mice (n=8) and Wild-Type (WT) mice (n=11) received two sets of non-invasive right unilateral cVNS (AM and PM) through GammaCore, each set consisting of two 2-minute stimulations with a 3-minute interval in between (20V; 60mA; 25Hz; 1ms bursts of 5kHz sine waves). Behavioral activity was assessed using Open Field Testing (OFT) and EthoVision XT software, and cerebral blood flow (CBF) was measured using laser speckle imaging (LSI), both pre- and post-cVNS, to evaluate effects on cognitive habituation and cerebral hemodynamics. Also, CBF changes were recorded during one cVNS set on the next day. Compared to baseline, two sets of non-invasive cVNS induced pronounced cerebral autoregulation and habituation in FAD mice compared to WT. Rapid return to baseline CBF from isoflurane-induced hyperperfusion was observed in FAD mice, with a linear regression slope of -197 ($r^2 = 0.0368$, $p < 0.0001$). Furthermore, during the 3-minute interval, FAD mice exhibited less CBF decline,

showing more stability ($p < 0.05$). Behavioral analysis of locomotor activity (mean distance moved) using OFT revealed significant reductions in distance traveled in FAD mice post-VNS; pre- and post-VNS locomotor activity significantly decreased between 2-3 minutes ($t = 3.52$, $p=0.0098$) and 7-8 minutes ($t = 3.23$, $p = 0.0145$). Our findings suggest that cVNS improves CBF autonomic regulation and habituation. cVNS-mediated effects on CBF autoregulation, critical in AD pathology, may serve as one mechanism for cognitive enhancements in AD models.

Disclosures: B. Chung: None. **A. Mohtasebzada:** None. **S. Berduo:** None. **K. Suayngam:** None. **R. Lotfy:** None. **M. Rafi:** None. **S. Khatami:** None. **S. Andalib:** None. **Y. Akbari:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.11/E32

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

Support: 5R01NS034239-28

Title: Combinatorial therapy with low dose IL-2 & Amyloid Beta targeting antibody for Alzheimer's disease

Authors: *R. KADRY[1], S. BHATTARAI[2], E. FOSTER[2], S. SRIVASTAVA[2], Y. LU[2], R. MOSLEY[2], P. YEAPURI[2], H. E. GENDELMAN[2];
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Abstract: Alzheimer's disease (AD), the most common form of dementia, is characterized by the accumulation of neurotoxic misfolded amyloid-beta (Aβ) protein, leading to neuronal loss and cognitive impairment. Soluble oligomeric Aβ (oAβ) isoforms are the most neurotoxic isoforms, which eventually aggregate into insoluble plaques. Passive immunization using monoclonal antibodies (mAbs) with enhanced affinity for oAβ has shown promise in clearing plaques and enhanced cognitive function. However, clinical outcomes have been mixed, partly due to antibody mediated proinflammatory responses, leading to Amyloid related imaging abnormalities-edema (ARIA-E). Antibody triggered immune activation can shift microglia toward a proinflammatory phenotype and induce neuroinflammatory effector T-cell (Teff) responses. Thus, there is an urgent need for mAbs highly selective for oAβ with minimized neuroinflammatory immune activation. Our hypothesis is that combining a novel oAβ selective mAb with a regulatory T-cell (Treg) stimulating agent, such as low dose Interlukin-2 (IL-2), will enhance noninflammatory pathway for amyloid clearance and reduce adverse neuroinflammatory effects. Molecular dynamics simulation and computational modeling were employed to identify unique epitope sequence presented on oAβ isoform. Mice were immunized with the epitope, and splenocytes were fused with P3/NSI/1-AG4-1 cells to generate hybridomas. Hybridoma

monoclones were screened via immunoprecipitation and surface plasmon resonance (SPR) for selective binding to Aβ isoforms (monomer, oligomer, fibril). Our novel mAb demonstrated higher selectivity for neurotoxic Aβ isoforms compared to FDA approved mAbs, Aducanumab and Lecanemab. Therapeutic efficacy of the combinatorial therapy was evaluated in APP/PS1 transgenic mice treated with mAb alone, IL-2 alone or in combination with low dose IL-2. Compared to mAb alone and IL-2 alone, combinatorial therapy significantly reduced brain infiltrating inflammatory Teff cells, improved cognitive function (Radial arm water maze), and decreased amyloid burden compared to mAb alone and IL-2 alone treatment groups. These results support a translational approach combining oAβ specific mAb therapy with Treg stimulating agents like IL-2 to achieve effective amyloid clearance with reduced neuroinflammatory side effects, offering a promising strategy to improve the safety and efficacy profiles of immunotherapy in AD.

Disclosures: R. Kadry: None. **S. Bhattarai:** None. **E. Foster:** None. **S. Srivastava:** None. **Y. Lu:** None. **R. Mosley:** None. **P. yeapuri:** None. **H.E. Gendelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Exavir Therapeutics, Inc. F. Consulting Fees (e.g., advisory boards); Longevity Biotech.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.12/E33

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

Support: R37NS033123
R56NS33123
R35NS127253
R21NS128630
R21NS127028
R01NS137233
R01NS097903

Title: Overabundance of the stress granule component STAU1 in 5XFAD mice: a novel target for Alzheimer’s disease

Authors: *S. PAUL[1], J. WILKERSON[2], W. ERCANBRACK[1], K. FIGUEROA[1], W. HOLLAND[2], D. R. SCOLES[1], S. M. PULST[3];
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Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, development of amyloid-β (Aβ) plaques, and neurofibrillary tangles. The

5XFAD mouse model, harboring five familial AD mutations in the amyloid precursor protein (APP; Swedish: K670N/M671L, Florida: I716V, London: V717I) and presenilin-1 (PSEN1; M146L, L286V), develops Aβ accumulation by 1.5 months, amyloid plaques and gliosis by 2 months, cognitive deficits by 4 months, and neuronal loss by 6 months, providing a robust platform for studying AD pathogenesis. RNA-binding proteins and autophagy dysregulation are emerging contributors to neurodegeneration. Staufen1 (STAU1), an RNA-binding protein regulating mRNA transport, stability, translation, and stress granule formation, is implicated in spinocerebellar ataxia type 2 (SCA2) through interactions with ataxin-2 (ATXN2) and mTOR signaling, which disrupts autophagy. Similarly, TAR DNA-binding protein 43 (TDP-43) is associated with AD, limbic-predominant age-related TDP-43 encephalopathy (LATE), and other neurodegenerative disorders due to its role in RNA metabolism. We investigated protein expressions in cortex (frontal lobe) and hippocampus of 6 months old 5XFAD and wild type mice using semi-quantitative Western blot analysis. Notably, we report for the first time a significant overabundance of STAU1 in both brain regions of 5XFAD mice, suggesting a novel role in AD pathology. STAU1 overabundance showed fold changes of ~2.6 ±0.5 in the cortex and 2.3 ±0.4 in the hippocampus, similar to the results for ALS/FTD-BAC-*C9ORF72* mouse cortex tissues (4 months old). Glial fibrillary acidic protein (GFAP) was significantly elevated, indicating astrogliosis, while choline acetyltransferase (ChAT) and neuronal nuclear antigen (NEUN) levels were reduced, reflecting impaired cholinergic function and neuronal loss. TDP-43 and ATXN2 were overabundant, indicating altered RNA metabolism, while nucleolin (NCL) levels were decreased and phosphorylated state of eukaryotic elongation factor 2 (P-EEF2) was increased, suggesting aberrant RNA processing and protein synthesis. Our data also reveals the overabundance of mTOR and p62, indicating autophagy dysregulation in 5XFAD mice. STAU1's overabundance, linked to mTOR hyperactivation and impaired autophagy in neurodegeneration, suggests its association in AD pathology by disrupting RNA metabolism and protein clearance. These findings support the notion that reducing STAU1 levels by gene-targeting approaches could restore autophagic balance and mitigate AD progression, warranting further mechanistic and therapeutic studies.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.13/E34

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

Support: NIH/NIA AG072510

Title: Proteasomal phosphorylation attenuates oxidized proteins in 5xFAD mice

Authors: *A. CHAKROBORTY[1,2], O. ADENIJI[2,1], A. A. DWAMENA[2,1], E. A. GILSTRAP[2,1], F. GORJIPOUR[1,2], Y. ASADI[2,3], J. STERNBURG[4], X. WANG[5], H. WANG[2,1];
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Abstract: Alzheimer's Disease (AD) is clinically diagnosed as persistent decline of behavioral and cognitive functions, that are coupled with manifestations of amyloid β-protein (Aβ) plaques and neurofibrillary tangles of either mutated or hyper phosphorylated tau protein in brain. As the disease progresses, brain tissues show increased accumulation of toxic proteins due to a consequence of elevated oxidative stress. The proteasome is a major protein degradation machinery in the cell to remove damaged proteins. Previous data have indicated that phosphorylation of the proteasome subunit PSMD11 (or Rpn6) at Ser14 enhances proteasomal activity and reduces misfolded protein-caused cytotoxicity in cell cultures; however, this has not been tested in the brain. In this study, we have investigated the level of oxidized proteins, aging markers and formation of γH2AX in 5xFAD mice either with PSMD11/Rpn6-Ser14 phosphorylation-blockage (S14A) or phosphorylation-mimicry (S14D) and compared with the 5XFAD mice. Additionally, 5XFAD and heterozygous S14A::5XFAD mice were subjected for treatment with Forskolin (Fsk, 5 mg/kg/day), an activator of adenylyl cyclase to increase phosphorylation of PSMD11. After the treatment, the animals were humanely sacrificed, and the brains were harvested to determine neuropathology. Our results showed that Fsk treatment reduces the level of oxidized proteins in the 5XFAD mouse brain compared to S14A::5XFAD brain. Compared to the 5XFAD harboring phosphorylation-mimicry PSMD11 (i.e. 5XFAD::S14D/D) mice, 5XFAD mouse brains showed relatively elevated level of oxidized proteins. Moreover, the month long intervention also improved cognitive behavior of 5XFAD mice in a PSMD11-Ser14 phosphorylation-dependent manner, as assessed by Radial Arm Water Maze test.

Disclosures: A. Chakroborty: None. **O. Adeniji:** None. **A.A. Dwamena:** None. **E.A. Gilstrap:** None. **F. Gorjipour:** None. **Y. Asadi:** None. **J. Sternburg:** None. **X. Wang:** None. **H. Wang:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.14/E35

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

NIH Grant GM-56647

Title: Investigating the Neuroprotective Role of Estrogen Receptors in Alzheimer's Disease in Down syndrome: A Postmortem Analysis

Authors: *F. GONZALEZ[1], E. HEAD[2];
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Abstract: Nearly all people with Down syndrome (DS) develop Alzheimer's disease (AD), a devastating neurodegenerative disorder, however, it is not clear how biological sex impacts disease progression. Women with DS experience menopause, characterized by a large drop in estrogen levels, around 5-7 years earlier than women in the general population. Estrogen receptor alpha (ERα) is activated by estrogen binding causing them to dimerize and translocate to the nucleus from the cytoplasm, where they activate transcription. We hypothesized that ERα levels would negatively correlate with AD pathology and that women with DSAD (Down Syndrome individuals with Alzheimer's disease) would have reduced nuclear ERα compared to men with DSAD.
Using human postmortem brain tissue (frontal and occipital regions), we performed immunohistochemistry for AD markers (Aβ, Tau) and ERα, and quantified them with digital pathology software (QuPath). Women with DSAD showed a positive correlation between ERα and both Aβ and Tau, while men with DSAD exhibited a positive correlation between ERα and Aβ but a negative correlation with Tau. Western blot analysis revealed higher active (dimerized) ERα in DSAD men cytoplasmic and nuclear fractions compared to women with DSAD in the occipital cortex.
Our findings suggest that ERα signaling may be impaired in DSAD women, contributing to AD progression. Future studies targeting ERβ and other brain regions are needed to inform therapeutic strategies.

Disclosures: F. Gonzalez: None. **E. Head:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.15/E36

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

Support: R21-MH121723-S1.

Title: Retinal Regulation of Locus Coeruleus: A Chemogenetic Approach to Treat Neurodegenerative Disorders

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Abstract: Alzheimer's Disease (AD) is the most common form of dementia, with early pathology linked to tau protein accumulation in the locus coeruleus (LC). Although direct LC activation has been shown to rescue cognitive deficits in animal models [1], its deep brainstem location limits clinical applications. The suprachiasmatic nucleus (SCN) indirectly influences LC

via the dorsomedial hypothalamus (DMH) [2], being in a key position to integrate light information with LC, via a circuit we denote as the Photic Regulation of Arousal and Mood (PRAM) pathway (retina → SCN → DMH → LC) [3]. This study tested whether PRAM activation could mitigate cognitive deficits in the Tg-F344 AD rat model. Six-month-old Tg-F344 and WT rats received intravitreal injections of an AAV encoding an excitatory Gq DREADD or control virus. Learning and memory were assessed three months later using the Morris Water Maze (MWM). DREADD activation via clozapine-N-oxide (2 mg/kg, i.p.) was administered 30 minutes before acquisition and/or referral, and reversal sessions. Electrophysiological and Fos analyses confirmed increased activity in retinal ganglion cells, SCN, DMH, and LC without impairing visual acuity. Notably, only female Tg-F344-AD rats exhibited initial learning deficits at 9 months, which were improved by six days of retinal DREADD stimulation. PRAM activation also enhanced memory recall during referral. Interestingly, PRAM activation during acquisition influenced reversal learning (i.e., learning a new location of the platform). Rats with PRAM stimulation during acquisition showed impaired reversal performance later, potentially due to enhanced memory of the original platform location (which could interfere with learning a new location). Using structural multigroup equation modeling (SEM), we confirmed that acquisition-phase PRAM stimulation increased escape latencies during reversal. However, adjusting for this acquisition effect using centered regression prediction analysis revealed a beneficial impact of PRAM activation during reversal trials. These data indicate that PRAM stimulation during reversal improves cognitive flexibility, but its effects can be masked by prior acquisition-phase stimulation. These findings suggest that PRAM-induced LC activation can attenuate AD-related learning deficits. As a relatively non-invasive approach, PRAM modulation may offer a promising therapeutic avenue for AD and other neuropsychiatric conditions linked to LC dysfunction. Supported by PHS grant R21-MH121723-S1. [1] Rorabaugh et al. 2017 [2] Aston-Jones et al. 2001 [3] Bowrey and Aston-Jones 2017

Disclosures: S. Delcourte: None. **G. Crozier:** None. **G.S. Aston-Jones:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.16/E37

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

Support: NIH Grant AG083079
Grant from the Carol and Gene Ludwig Family Foundation

Title: Genetic and antibody-mediated enhancements of TREM2 activity ameliorate Alzheimer's disease-related network hyperexcitability and brain dysfunction

Authors: *V. HUANG[1,2], M. DAS[1,2], G.-Q. YU[1,2], W. XIONG[3], N. ZHANG[3], Z. AN[3], L. MUCKE[1,2];
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Abstract: Alzheimer's disease (AD) can be associated with nonconvulsive epileptiform activity, and this network hyperexcitability predicts faster cognitive decline. Recent studies have identified intriguing pathogenic links between AD-related neural networks and immune cell dysfunctions. For example, hypofunction of the triggering receptor expressed on myeloid cells 2 (TREM2) increases AD risk in humans and susceptibility to epileptiform activity in related mouse models. These findings raise the question of whether enhancing the level or activity of TREM2 might suppress network hyperexcitability and related brain dysfunctions. Here, we used transgene-mediated overexpression and treatment with an agonist TREM2 antibody to show in mouse models that increasing the level or activity of TREM2 suppresses acute, excitotoxin-induced seizures and chronic nonconvulsive epileptiform activity caused by amyloid precursor proteins bearing autosomal dominant Alzheimer's disease mutations. In the latter model, TREM2 antibody treatment also reversed deficits in learning and memory. These results suggest that TREM2 signaling allows microglia to rapidly counteract network hyperexcitability and that this function can be augmented and leveraged for therapeutic purposes. Enhancing TREM2 functions with agonist antibodies or small molecules may help engage microglia in suppressing aberrant neuronal activities that disrupt important brain functions in disorders as diverse as Alzheimer's disease, epilepsy, and neuropsychiatric conditions.
References: Das et al., iScience 24: 103245 (2021); Das et al., Neurobiol. Dis. 186: 106263 (2023); Zhao et al., Sci. Transl. Med 14: eabq0095 (2022)

Disclosures: V. Huang: None. **M. Das:** None. **G. Yu:** None. **W. Xiong:** None. **N. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of PCT application 18/691,229, TREM2 Antigen Binding Proteins and Uses Thereof, University of Texas Health Science Center at Houston. **Z. An:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of PCT application 18/691,229, TREM2 Antigen Binding Proteins and Uses Thereof, University of Texas Health Science Center at Houston. **L. Mucke:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.17/E38

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

Title: Single-cell genetic dissection to unravel the role of genotoxic stress in alzheimer's disease onset and progression

Authors: *C. HAACKER;
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Abstract: Alzheimer's disease (AD) lacks effective disease-modifying therapies due to poorly understood etiology and progression. Our prior work identified age-dependent somatic mutation accumulation in the brain in several neurodegenerative disorders, prompting investigation into DNA damage's role in AD pathogenesis. Using triple transgenic AD mice and a novel single-cell genotoxic stress sensor (PRISM), we observed age-dependent increases in brain DNA damage (γH2AX) exceeding age-matched controls, with regional correlations to amyloid-beta and tau pathology. To model genotoxic stress, we employed the environmental toxin methylazoxymethanol (MAM), known to induce neurodegenerative phenotypes. Differentiated human neurons exposed to MAM showed elevated DNA damage sensor expression, cellular senescence markers (p21), and neuroinflammation (NF-κB, Iba1). These findings were replicated *in vivo*, with intra-striatal MAM administration increasing γH2AX, senescence, and pathogenic tau—directly linking genotoxic stress to disease pathology. We also developed a pre-formed fibril (PFF) co-fibril [αSyn+Tau] model of ADRD progression. Utilizing our genetic sensor and actuator of DDR with cre-dependable conditional shRNA, knockdown of ATM only occurred in neurons exhibiting DNA damage. Additional treatment groups included small-molecule pharmacological ATM inhibition. Treatment groups rescued behavioral deficits and pathological hallmarks, demonstrating causal involvement of DNA damage response (DDR) pathways. Notably, we observed nuclear localization of pathologic phospho-tau or phosphor-synuclein within enlarged DNA damage foci in transgenic AD mice and PFF progression model, respectfully. Real-time tracking of GFP-fused synuclein after DNA damage in differentiated human neurons together with AlphaFold protein-interaction modeling, suggest genotoxic stress-driven nuclear shuttling of ATM may phosphorylate and recruit tau and synuclein to DNA damage foci to exert long-term impact on neuronal fate and function. Collectively, our work establishes genotoxic stress as a causal driver of ADRD progression. By integrating genetic, toxin-based, and induced pathological models, we provide a framework for mechanistic pathogenesis of ADRD and development of DDR components as actionable therapeutic drug targets.

Disclosures: C. Haacker: None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.18/E39

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

Support: R35ES035043-01
P20GM103408
P20GM109095

Title: Exercise-induced stress in transgenic Alzheimer's disease rats causes sex-specific changes in amyloid burden, glial phenotype, and epigenetic patterning in the hippocampus

Authors: *M. R. HAGER[1], A. SCHULLER[1], S. ROCHA[1], A. BRIGGS[1], S. HALL[2], R. B. TJALKENS[1];
[1]Envrn. and Radiological Hlth. Sci., Colorado State Univ., Fort Collins, CO; [2]Kansas State Univ. Col. of Vet. Med., Manhattan, KS

Abstract: Aerobic exercise has been shown capable of altering the progression of Alzheimer's disease (AD) in both animal models and in humans. Although physical activity throughout life can be protective against neurodegeneration, limited work has been done to characterize the effects of a robust exertion regimen implemented after the onset of disease. We therefore explored pathologic protein burden, glial phenotype, and DNA modification profile of the hippocampus in TgF344 rats subjected to progressive treadmill training from 12 to 16 months of age. Hippocampal sections were immunofluorescently labeled, imaged via high-content scanning microscopy, and automatically quantified using deep learning-based neural networks trained across the dataset. There was a female-specific reduction in $NeuN^+$ neurons/mm^2 in the dentate gyrus and $Iba1^+$ microglia/mm^2 across the entire hippocampal extent following physical exertion in aged animals. Neither sex exhibited changes in the percent of $GFAP^+$ area across the hippocampus by treatment group. 3D rendering and morphologic assessment of plaque-associated microglia revealed a more robust disease-associated phenotype in female animals following exercise-induced stress. Total amyloid-ß occupied area, but not phospho-TauTh181 (pTau) burden, varied significantly by both exercise and sex status, increasing in females subjected to activity stress but decreasing in males. Epigenetic profiling via reduced representation bisulfite sequencing identified 94 genes associated with DMCs in physically exerted females (355 DMRs, 54.6% hypermodified) and 87 genes associated with DMCs in treadmill trained males (313 DMRs, 50.4% hypermodified). Of those, 13 genes overlapped between sexes, with 3 being concordantly modified: *Insl3*, *Fgfr2*, and *Gnas*. Together, these results indicate unique, sex-specific hippocampal responses to physical exertion in aged AD transgenic rats and broadly suggest the importance of intensity and timing of onset when considering exercise as a potential disease-modifying intervention.

Disclosures: M.R. Hager: None. **A. Schuller:** None. **S. Rocha:** None. **A. Briggs:** None. **S. Hall:** None. **R.B. Tjalkens:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.19/E40

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

Title: Funding and Design Factors Influencing the Discontinuation of Alzheimer's Clinical Trials: A 20-Year Review

Authors: *A. HOZAN, G. BAHOO, A. MCMURRAY, M. GACKLE, J. ASHURST; Midwestern Univ., Glendale, AZ

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease with a growing public health burden, affecting over 7 million individuals in the USA alone. Despite decades of research, treatment is still limited, and clinical trials are crucial in the pursuit of effective disease-modifying therapies. This investigation aims to explore the features of terminated and withdrawn AD trials to reveal patterns and inform future trial sustainability and design. A retrospective cohort of clinical trials about AD from 12/01/2013-04/01/2025 were reviewed from clinicaltrials.gov. Trial data collected included the status, intervention type, trial phase, funding source, study type, reason for discontinuation, publication of results, and publication status. Categorical variables were analyzed using a chi-squared test and are presented as a count. A total of 362 clinical trials were reviewed with 276 being terminated and 86 being withdrawn. Most studies reviewed were randomized (52.5%) drug (56.1%) studies that were privately sponsored (59.1%). Most studies were discontinued due to either safety/efficacy (24.0%) or recruitment/enrollment (23.5%) issues. Clinical drug trials that were privately sponsored (66.67%) were more likely to be terminated while drug trials that were academically sponsored (59.3%) were more likely to be withdrawn. Safety/efficacy (30.8%) was the main reason to terminate a study while recruitment/enrollment (30.2%) was the main reason to withdraw a study. A statistical difference was noted between the intervention type ($p<0.001$), trial phase ($p<0.001$), funding source ($p<0.001$), sponsor ($p<0.001$), and reason for discontinuation ($p<0.001$). Statistically significant differences were observed in intervention type, trial phase, sponsor, and funding source. The difference between sponsor and trial phase suggests patterns that may leave certain types of trials more vulnerable to discontinuation depending on the sponsor.

Disclosures: A. Hozan: None. **G. Bahoo:** None. **A. McMurray:** None. **M. Gackle:** None. **J. Ashurst:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.20/E41

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

Support: Indian Council of Medical Research (ICMR: funding number 2020–1194)
Department of Health Research (DHR: GIA/2020/000595)
Council of Scientific and Industrial Research (CSIR: 09/0006(0519)/2019-EMR-I)

Title: Stress-mediated Changes in Extracellular Vesicles Scaffold Sequesters Small Amyloid-β Aggregates: A Novel Insight Into Alzheimer's Disease Progression

Authors: *S. RAI, S. ZEHRA, S. KUMAR;
Biophysics, All India Inst. Of Med. Sciences, New Delhi, New Delhi, India

Abstract: Introduction: Despite extensive research on the role of extracellular vesicles (EVs) in Alzheimer's disease on Aβ transport and plaque formation, underlying mechanisms remain unclear. This study evaluates the EVs' association with different Aβ aggregates and examines stress-induced alterations in EVs' membranes and their association with Aβ aggregates.
Methods: EVs were isolated by size exclusion chromatography, and different-sized amyloid-β aggregates were prepared to assess their association with EVs. Characterization was conducted using Transmission electron microscopy (TEM), Confocal microscopy (CFM), Western blotting, Nanoparticle tracking analysis, Dynamic light scattering, and Thioflavin-T assay. EVs-Aβ association was analyzed using TEM, CFM, and Direct stochastic optical reconstruction microscopy (dSTORM) with fluorescently labeled antibodies.
Results: TEM, CFM, and dSTORM images showed that EVs had a higher affinity for small amyloid-β aggregates (SA) over un-aggregated (UA) and large aggregates (BA). We observed the EV-Aβ association under mechanical (ultrasonication, mild agitation), physical (temperature) stresses, and biological (Oxidative stress), which induced structural reconfiguration of sEVs, enabling selective high-affinity binding to Aβ aggregates. Using super-resolution imaging and quantitative colocalization assays, we provide compelling evidence that stress-altered sEVs function as "pathological chaperones," enhancing neuronal internalization of small Aβ aggregates (SA) in vitro. Importantly, EVs-Aβ aggregates were observed around amyloid-β plaques in post-mortem Alzheimer's disease (AD) brains and the brains of APP-PS1 mice. Furthermore, EVs from healthy controls showed no association with SA, while EVs from AD patients (AD-EVs) showed a significant association, establishing a link between experimental findings and human pathology. Comparative Lipidomic analysis of AD-EVs, EVs exposed to oxidative stress (hEVs), and untreated EVs (nEVs) with SA revealed that dysregulated lipid classes such as Lysophosphatidylcholine (LPC), Sphinganine (SPB), and Ceramide (Cer) were highly dysregulated in hEVs compared to nEVs, and lipid levels in the AD-EVs closely mirrored those observed in hEVs.
Conclusion: This work challenges long-held assumptions about the passive role of EVs in Aβ trafficking, instead revealing a dynamic, stress-dependent mechanism that alters the EV membrane, promoting preferential Aβ-SA sequestration. This study highlights the role of altered EV dynamics in AD and reshapes our understanding of Alzheimer's disease (AD) progression.

Disclosures: S. Rai: None. **S. Zehra:** None. **S. kumar:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.21/E42

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

Support: U01AG074960

Title: Behavioral and Neurochemical Abnormalities in Humanized Tau-FAD Combination Mice

Authors: *K. SAMBAMURTI[1], P. PADHI[2], M. SAMIDURAI[4], M. A. PAPPOLLA[5], D. K. LAHIRI[6], N. GREIG[7], A. G. KANTHASAMY[3];
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Abstract: Familial Alzheimer's disease (FAD) mouse models expressing mutant amyloid precursor protein (APP) and presenilin-1 (PS1) have been critical for understanding amyloid β (Aβ) pathology. However, most models do not permit independent evaluation of Aβ and Tau contributions, as the pathologies often co-occur in tightly linked transgenes. To address this gap, we generated two novel strains by crossing hTau mice (JAX #005491), which express human wild-type genomic *MAPT* and lack mouse *Mapt*, with APP/PS1 (HTAP, MMRRC #034833-JAX) or 5xFAD (HTAP2, MMRRC #034848-JAX) mice. These new models enable the separate but concurrent study of human Tau- and Aβ-driven pathologies on a defined C57BL/6J background. We evaluated spatial memory using the radial arm water maze and Barnes maze, measured norepinephrine levels in the hippocampus, cortex, and striatum by HPLC, and assessed microglial activation via IBA1 immunohistochemistry. Both HTAP and HTAP2 mice exhibited Aβ plaque deposition patterns similar to those of their parental FAD lines and expressed high levels of human Tau with abnormal hyperphosphorylation by 7 months. Compared to wild-type, hTau-only, or FAD-only controls, both HTAP strains exhibited significantly impaired spatial memory. Notably, HTAP2 mice showed markedly reduced norepinephrine concentrations and elevated IBA1-positive microglial burden in hippocampal and cortical regions. These findings suggest that the co-expression of human Tau and aggressive Aβ pathology leads to exacerbated behavioral deficits, catecholaminergic depletion, and neuroinflammation. The HTAP and HTAP2 models offer translational relevance for dissecting the interplay between Tau and amyloid in AD, providing a platform for investigating microRNAs that regulate MAPT via its 3' UTR, as well as therapies targeting neuroimmune and monoaminergic pathways.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.22/E43

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

Support: R01 NS128053

Title: Bmp inhibition improves hippocampus-dependent cognition and cellular changes in multiple tauopathy models

Authors: *J. GEIER[1], A. AFFANEH[2], Y.-P. LI[3], J. ALLEN[4], C.-Y. PENG[3], J. A. KESSLER[3]; [1]Davee Dept. of Neurol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Neurol., Washington Univ. in St. Louis, St. Louis, MO; [3]Neurol., [4]Northwestern Univ., Chicago, IL

Abstract: Tauopathies, defined by the pathological accumulation of hyperphosphorylated tau protein in neurons and glia, are a central pathological feature of multiple neurodegenerative diseases, including Alzheimer's disease (AD), frontotemporal dementia (FTD), and progressive supranuclear palsy (PSP). Aging is the strongest and most consistent risk factor across these disorders, implicating age-related molecular pathways in disease progression. Our lab and others have identified a robust, age-dependent increase in bone morphogenetic protein (BMP) signaling within the hippocampus of both humans and mice. Notably, our prior work demonstrated that elevated BMP activity enhances tau phosphorylation and secretion in patient-derived induced neurons and astrocytes (iNs) from both APOE3 and APOE4 backgrounds, implicating BMP signaling as a potential driver of tau pathology in the aging brain.To assess the in vivo relevance of these findings, we overexpressed Noggin, a well-characterized endogenous BMP antagonist in the mouse brain. BMP inhibition led to significantly improved hippocampus-dependent learning and memory in two distinct tauopathy mouse models, as assessed by Barnes Maze performance: (1) P301S mice received dentate gyrus-targeted lentiviral Noggin expression, and (2) transgenic APOE4 x NSE-Noggin mice constitutively overexpressed Noggin in neurons. In APOE4 x NSE-Noggin mice, hippocampal phospho-tau levels were reduced, and Fluoro-Jade staining showed decreased neurodegeneration relative to APOE4 controls. These results support a beneficial role for BMP pathway inhibition in mitigating tau pathology and preserving cognitive function.To expand the translational potential of this approach, we are currently testing BMP inhibition in additional models of neurodegeneration, including aged 5xFAD mice. Collectively, our data suggest that BMP inhibition may represent a novel and potentially promising therapeutic strategy for tau-driven cognitive decline in aging and neurodegeneration.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.23/E44

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

Support: Dementia Australia Research Foundation project grant
Western Sydney University Hands-on Research equipment grant
Western Sydney University Collaborative project grant

Title: Turn up the heat: sauna-like treatment rescues behavioral deficits in a mouse model of Alzheimer's disease

Authors: *R. ROSA PORTO[1], B. SMITS[1], J. COLEMAN[1], I. JAJJO[1], O. SOLIER[1], G. PEREIRA[1], M. PUVVENA[1], T. KARL[2];
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Abstract: Alzheimer's disease (AD) therapy is still limited to only modest and temporary improvements and does not halt or reverse the underlying pathology. Heat treatment (e.g. sauna) has been found to improve physical and mental health in chronic diseases, such as cardiovascular disease, diabetes and depression. Most importantly, there is an inverse correlation between sauna frequency and AD. Our aim was to evaluate the remedial therapeutic properties of a clinically relevant heat treatment (HT) protocol in AD transgenic mouse model thereby also considering sex effects. We used 6-month-old female and male TAU58/2 transgenic mice (hyperphosphorylated tau model), along with respective wildtype-like (WT) littermates (n = 7-14). HT protocol was validated with acute and chronic pilots where a 20-minute exposure to 42°C promoted an increase in the levels of the heat shock protein of 70 kDa (HSP70) in the hippocampus and prefrontal cortex of C57BL/6 mice. Therefore, this protocol was applied twice weekly for a period of 8 weeks in TAU58/2 mice. Controls were exposed to room temperature (24°C). Subcutaneous body temperature and behavior were recorded every minute during the first and final HT sessions. Body weight and general health were assessed pre- and post-treatment. Starting two days after the final HT, AD transgenic mice were tested in an array of behavioral paradigms to evaluate HT effects including open field (locomotion), novel object location (spatial memory), elevated plus maze (anxiety), social preference (sociability and social recognition memory), prepulse inhibition (sensorimotor gating), beam walk and accelerod (motor performance). HT significantly increased body temperature of all mice (> 3°C from baseline) without any adverse health effects. Body weight was reduced in the TAU58/2 but there was no effect of treatment. AD mice showed motor impairments; however, HT improved motor performance in males and females in the beam walk and in males in the accelerod test. Males that underwent HT also had increased locomotion in the open field, with increased speed and distance travelled. Spatial memory deficits in the TAU58/2 males were rescued by HT. TAU58/2 mice exhibited impaired startle response and prepulse inhibition, as well as reduced anxiety levels in the elevated plus maze, with no pronounced effects of HT. Social behaviors remained intact across all groups. These results validate a non-pharmacological intervention that mitigates cognitive and motor impairments in AD-relevant model at symptomatic stages. HT was safe, effective, and potentially translatable to human clinical use.

Disclosures: R. Rosa Porto: None. **B. Smits:** None. **J. Coleman:** None. **I. Jajjo:** None. **O. Solier:** None. **G. Pereira:** None. **M. Puvvena:** None. **T. Karl:** None.

Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.24/E45

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

Support: NIH NIA F30 AG084348
NIH NIGMS U54 GM115458
Fremont Area Alzheimer's Fund

Title: Repetitive transcranial magnetic stimulation in mild amnestic cognitive impairment: effects on memory and brain variables

Authors: *A. HELLER-WIGHT, C. J. PHIPPS, H. GHANNAM, A. WILHELM, E. A. ARMBRUSTER, V. PHATAK, D. L. MURMAN, D. E. WARREN;
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Abstract: Alzheimer's Disease (AD) and its frequent prodrome, amnestic mild cognitive impairment (aMCI) are pathological aging processes that significantly impact brain structures such as the hippocampus that are necessary for normal memory function. Current interventions for clinical memory impairment slow disease progression, but do not restore memory abilities. Repetitive transcranial magnetic stimulation (rTMS) is a novel, noninvasive therapy that has previously been shown to improve memory ability in younger and older adults. Stimulation of the angular gyrus (AG), a brain region functionally connected to the hippocampus, may confer significant brain and cognitive benefits for patients with clinical memory impairment. The current study aimed to test this hypothesis by administering rTMS to patients diagnosed with aMCI using a double-blind, intent-to-treat, randomized controlled trial (ClinicalTrials.gov #alz078533). Enrolled individuals (N = 15) completed two five-day sessions of rTMS. The treatment session was one week of β-frequency pulse sequences which alternated 2-sec. 20 Hz stimulation with 28-sec. rest for 20 min (1600 pulses). The sham session was one week of stimulation with the same parameters at 10% of individual resting motor threshold. Stimulation was administered to each participant's area of left AG exhibiting peak resting-state functional connectivity (RSFC) with left hippocampus, and session order was counterbalanced. Cognitive and RSFC changes were assessed with linear mixed effect models, followed by paired t-tests. Results revealed significant changes in verbal memory, including number of correct responses on short-delay free recall ($\beta = 2.22$, df = 38.15, $t = 2.12$, $p = 0.02$, Intercept Term = 5.29) and semantic cluster scores of long-delay free recall ($\beta = 1.01$, df =38.14, $t = 1.80$, $p = 0.04$, Intercept Term = 1.67). There were also significant differences in RSFC. Decreased connectivity was observed between the left hippocampus and the prefrontal cortex (strict threshold $p < .001$, 13 voxels) and increased connectivity was observed between left hippocampus and right postcentral gyrus (exploratory threshold $p < .05$, 269 voxels). Patients with aMCI enrolled in the trial exhibited changes in brain and cognitive variables after treatment with rTMS, suggesting a potential cognitive benefit of rTMS in patients with clinical memory impairment. rTMS therapy has the potential to offer patients in prodromal stages of AD greater duration of persevered memory and improved AD treatment outcomes.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

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Program #/Poster #: PSTR382.25/E46

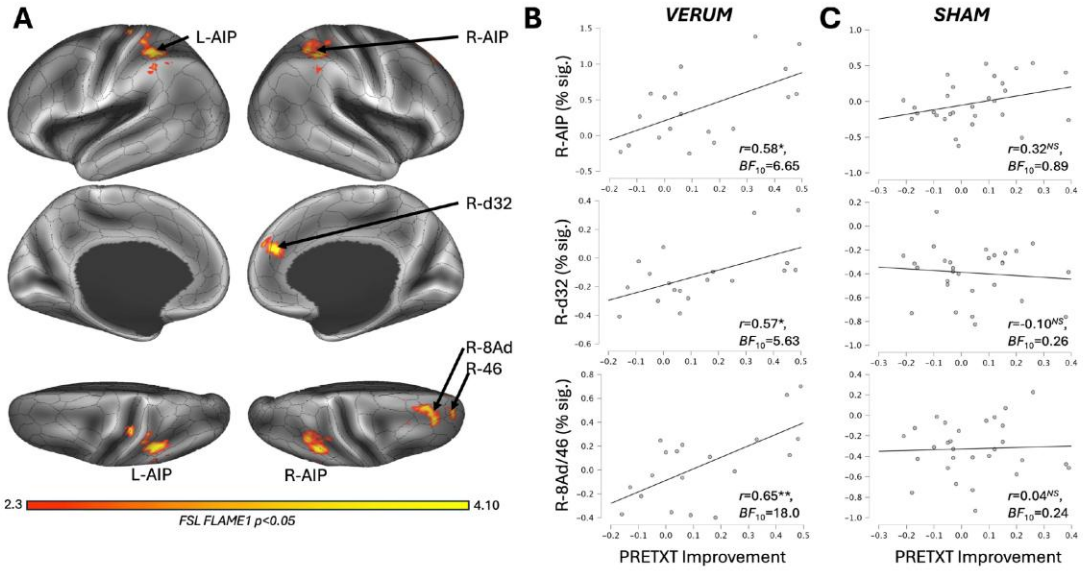
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 3P30GM122734-05S1

Title: Neuroimaging-guided transcranial direct current stimulation alleviates learning deficits in mild cognitive impairment with over 10-fold improvement through the modulation of frontoparietal networks: Results from the MEMORI randomized double-blind clinical trial

Authors: *V. P. CLARK[1], B. GIBSON[2], M. BRIGGS[3], J. HOGEVEEN[4], A. J. WOODS[5], J. P. PHILLIPS[6], S. G. RYMAN[7], M. COX[8], A. MAYER[9];
[1]Psychology Clin. Neurosci. Center, Dept. Psychology, [3]Psychology, [2]Univ. of New Mexico, Albuquerque, NM; [4]Dept. of Psychology, The Univ. of New Mexico, Albuquerque, NM; [5]Sch. of Behavioral and Brain Sci., The Univ. of Texas at Dallas, Richardson, TX; [6]Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [7]Translational Neurosci., Mind Res. Network, Albuquerque, NM; [8]Wexford Hlth. Services, Pittsburgh, PA; [9]The Mind Res. Network, Albuquerque, NM

Abstract: Alzheimer's disease (AD) and other dementias impose substantial personal and financial burdens. Prior neuroimaging-guided transcranial direct current stimulation (tDCS) studies from our laboratory have demonstrated 2-4 fold learning improvement in healthy young adults. This registered randomized double-blind trial (NCT05077826) tested whether tDCS improves learning in healthy older adults and those with mild cognitive impairment (MCI). Eighty-seven participants (mean 67.4 y.o., 44 female) were enrolled, including 58 healthy controls (HC) and 29 with MCI, verified using standardized assessments. Participants completed the PRETXT task requiring visual categorization through discovery learning (Gibson et al. 2020). Following baseline testing, participants received either verum/active (2.0 mA) or sham (0.1 mA) tDCS for 30 minutes during training with the anode placed over right inferior frontal gyrus (F10) and cathode on the left arm. Baseline and final testing occurred while 3T fMRI was acquired. This tDCS treatment produced significant learning improvements: sham yielded 3.7%±2.0% learning (final training block minus baseline) while verum resulted in 13.0%±2.7% (254% relative increase, Cohen's d=0.59, p=0.004) across all participants. In MCI participants alone, verum tDCS showed 1027% greater learning relative to sham (p=0.027). Verum MCI showed greater learning than sham HC (122% relative increase, n.s.). Learning correlated with modeled intracranial verum field strength (p=0.007). Active tDCS enhanced fMRI responses in bilateral frontoparietal networks, including anterior intraparietal and right dorsolateral prefrontal cortices (FWE corrected p<0.05). These fMRI differences correlated positively with behavioral improvements in verum but not sham groups. This randomized blinded trial demonstrated that tDCS improves learning in MCI to the same level as healthy controls. The effect size exceeds that found for medications typically prescribed for AD. In conclusion, tDCS shows potential as a promising non-pharmacological intervention for MCI & AD.



tDCS-specific modulation of BOLD changes. (A) Several brain regions that were recruited during the decision epoch of the PRETXT task demonstrated greater changes in BOLD signal from pre-to-post stimulation in the verum group relative to the sham group ($p_{\text{FLAME1}}<0.05$). These regions included a bilateral anterior intraparietal area (AIP), right dorsal area 32 (d32), and regions of right dorsolateral prefrontal cortex comprising (8Ad and 46). **(B)** The degree of pre-poststimulation change in each of these areas demonstrated credible to strong positive correlations with behavioral improvements on the PRETXT task in the verum stimulation group (all $BF_{10}\geq 3$), **(C)** none of which were correlated in the sham tDCS group.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.26/E47

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

Support: NIA R01-AG061188

Title: A novel CHIP variant reduces tau pathology in models of Alzheimer's disease

Authors: *M. R. BRYAN, III[1], C. OPLAND[2], T. J. COHEN[3];
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Abstract: The C-terminus of heat shock 70-interacting protein (CHIP) is an E3 ubiquitin ligase that plays a critical role in neuronal protein quality control by promoting the repair or degradation of misfolded proteins such as aggregated tau (MAPT). Genetic studies have demonstrated that loss of CHIP function leads to neurodegeneration and cognitive impairment. Consistent with this, we have shown that CHIP levels are markedly reduced in Alzheimer's disease (AD) patient brains, implicating CHIP loss as a contributor to dementia susceptibility. Our preliminary data identify a gain-of-function variant of CHIP, termed CHIP NP, that may promote tau clearance. CHIP NP suppresses tau pathology more effectively than wild-type CHIP in vitro and in mouse primary neuron models of tau aggregation. Although the precise mechanism of action of CHIP NP remains under investigation, we hypothesize that these novel CHIP variants hold promise as gene therapies to prevent tau pathology, restore neuronal function, mitigate cognitive decline, and enhance survival. To test this, we engineered brain-penetrant adeno-associated virus vectors (AAV PHP.eB) to express CHIP NP in mouse models of AD. Following intravenous injection, CHIP NP was broadly expressed across the hippocampus and cortex without observable kidney or liver toxicity. Notably, in a small pilot study, CHIP NP co-localized with neurons exhibiting phosphorylated tau pathology and reduced hippocampal p-tau levels by approximately 50% compared to controls. These findings suggest that CHIP NP-AAV crosses the blood-brain barrier, engages pathogenic tau, and mitigates tau pathology with minimal toxicity. Ongoing studies are examining effects on tau aggregation, amyloid beta accumulation, gliosis, and cognitive outcomes. Our work supports the development of AAV-mediated CHIP NP expression as a promising gene therapy strategy for AD and other tauopathies.

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Poster

PSTR382: Alzheimer's Disease Therapeutic Strategies: Novel Approaches

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR382.27/E48

Topic: I.07. Long-Term Memory

Support: NIH R01 MH087463
R00 AG 068306

Title: Leveraging Molecular Chaperones as Therapeutic Tools Against Alzheimer's Disease and Related Dementias

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Abstract: Consolidation of newly acquired experiences into persistent long-term memories relies on transcriptional events that occur in a precise spatiotemporal fashion in the hippocampus. In our prior work, we utilized the Visium spatial transcriptomic sequencing (10x Genomics) to elucidate molecular signatures across hippocampal subregions following spatial learning. Intriguingly, our analyses revealed discrete patterns of learning-dependent induction in genes encoding for the endoplasmic reticulum (ER) chaperones in distinct hippocampal subregions. Building on these findings, we performed single-nuclei transcriptomics to identify cell-type-specific induction of ER chaperone-encoding genes in the dorsal hippocampus upon learning. Our analyses revealed distinct and overlapping cell-type-specific signatures of learning-dependent ER chaperone induction. For instance, genes encoding for chaperones Hspa5 and Pdia6 were upregulated in the CA1 pyramidal neurons and in the dentate gyrus (DG), while *Sdf2l1* expression was selectively induced in the DG. We then demonstrated the functional relevance of our transcriptomic findings by being able to enhance long-term spatial memory in wild-type mice upon genetically enhancing the expression of the ER chaperone Pdia6 selectively in the excitatory pyramidal neurons of the dorsal hippocampus. Clinically extending these observations, we identified a marked downregulation of key ER chaperone-encoding genes, including *Pdia6*, in the dorsal hippocampus of transgenic mice expressing the P301L mutant form of human tau. The P301L transgenic mouse model was selected for this study as it phenocopies core features of Alzheimer's disease and related dementias (ADRD), including tau pathology and cognitive impairments. Remarkably, reinstating Pdia6 expression in the dorsal hippocampus of P301L mice ameliorated deficits in long-term memory and attenuated pathological tau accumulation. Together, our findings provide unprecedented molecular insights into the mechanistic basis of hippocampal memory consolidation and establish the ER chaperones as critical regulators of long-term memory. Moreover, given that activation of the protein folding machinery confers resilience against long-term memory deficits and tau pathology in ADRD, our work highlights the therapeutic potential of molecular chaperones in treating neurodegenerative disorders.

Disclosures: U. Mukherjee: None. **B. Basu:** None. **T. Abel:** None. **S. Chatterjee:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.01/E49

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

Title: Greater adherence to neurodegenerative risk reduction care alters blood biomarkers of neurodegenerative disease: A prospective, multiplatform biomarker study

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Abstract: This ongoing prospective study explores plasma protein biomarkers as tools for early detection and to evaluate the biological effectiveness of therapeutic interventions for Alzheimer's Disease (AD) and other Neurodegenerative Diseases (NDDs), including Parkinson's Disease (PD) and Lewy Body Dementia (DLB). Participants with a family history of NDDs and no or minimal neurological symptoms, along with healthy controls, were recruited from five sites in the US and Canada. As of June 2, 2025, 216 participants were recruited, with 111 having longitudinal assessments analyzed. Participants receiving preventive neurology or medical care were stratified by adherence to prescribed recommendations: "Intervention 1" (≥60% adherence) and "Intervention 2" (<60% adherence), with both groups compared to non-intervention healthy and AD controls. NDD risk reduction interventions included lifestyle changes, lipid-lowering agents (e.g., statins, ezetimibe), GLP1 receptor agonists, hormone replacement therapy, and SSRIs. NULISA plasma testing revealed significant changes in three of four key protein ratios (pTau217/Aβ42, pTau181/Aβ42, Oligo-SNCA/SNCA) for Intervention 1 and two ratios for (pTau181/Aβ42, Oligo-SNCA/SNCA) Intervention 2. Additionally, 33 individual biomarkers changed significantly in Intervention 1 and 24 in Intervention 2. Multi-modal interventions (lifestyle + recommended vitamins and/or supplements and/or drugs) showed the highest number of significant changes. Lumipulse testing showed significant differences in the pTau181/AB42 ratio, Aβ40, Aβ42, pTau181, and pTau217 in Intervention 1, and changes in the pTau181/AB42 ratio, Aβ40, Aβ42, and pTau181 in Intervention 2. Controls showed significant changes in Aβ40 and Aβ42, and AD Controls showed no significant changes. The study demonstrates that higher intervention compliance and multi-modal approaches produced more significant changes in protein markers compared to lower compliance. Multi-modal interventions were most effective. Novel alpha-synuclein markers also changed, potentially aiding in evaluating interventions for Lewy Body Dementia and Parkinson's disease, as well as identifying potentially amplifying factors for traditional AD pathology. These results highlight the utility of plasma biomarkers for early detection, risk stratification, and monitoring of preventive interventions in NDD, with potential to guide precision neurologic care.

Disclosures: C.M. Janney: A. Employment/Salary (full or part-time): Institute for Neurodegenerative Diseases (IND). **J.G. Westine:** A. Employment/Salary (full or part-time): Institute for Neurodegenerative Diseases (IND). **C. Brubeck:** 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.; Institute for Neurodegenerative Diseases (IND). **S. Olson:** A. Employment/Salary (full or part-time): Institute for Neurodegenerative Diseases (IND). **J. Dishary:** A. Employment/Salary (full or part-time): Institute for Neurodegenerative Diseases (IND). **S. Helfman:** A. Employment/Salary (full or part-time): Institute for Neurodegenerative Diseases (IND). **J. Lakis:** 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.; Institute for Neurodegenerative Diseases (IND). **L. Valois:** None. **H. Hristov:** 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.; Institute for Neurodegenerative Diseases (IND). **S. Murray:** A. Employment/Salary (full or part-time): Institute for Neurodegenerative Diseases (IND). **A. Seifan:** 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.; Institute for Neurodegenerative Diseases (IND). **P. Sisser:** None. **J.A. Melendez-Herencia:** None. **A.J. Barber:** 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.; Institute for Neurodegenerative Diseases (IND). **N. Clute-Reinig:** 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.; Institute for Neurodegenerative Diseases (IND). **P. Parthasarathy:** None. **C. Saperia:** None. **D. Angerbauer:** None. **K. Niotis:** 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.; Institute for Neurodegenerative Diseases (IND). **R. Isaacson:** Other; Institute for Neurodegenerative Diseases (IND).

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.02/E50

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

Title: Multiplex spatial proteomics and AI-powered image analysis reveal region-specific protein expression in alzheimer’s disease brain

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Abstract: Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by progressive cognitive decline. AD is pathologically defined by the presence of β-amyloid-containing plaques and phosphorylated tau containing neurofibrillary tangles. Traditional bulk tissue proteomics has provided valuable insights into disease-associated molecular changes but lacks the spatial resolution necessary to dissect the intricate cellular and regional heterogeneity of the AD brain. Spatial proteomics, a cutting-edge approach integrating high-resolution imaging with quantitative proteomics, offers a transformative avenue to map protein expression within the native tissue context. In this study, we employed high-plex spatial proteomic profiling using Leica's Cell DIVE Multiplexed Imaging Solution, in combination with IF/IHC-validated antibodies from Cell Signaling Technology (CST) on brain tissues from AD patients and controls. Our analysis revealed region-specific alterations in synaptic proteins, glial activation markers, and astrocytes, with distinct spatial patterns emerging across the precentral gyrus and cerebellum. Notably, we identified amyloid deposition and microglial reactivity, as well as proteomic gradients correlating with the progression of pathological markers of AD. Here we demonstrate multiplexed Cell DIVE imaging using a novel CST panel to probe AD brain. Integrating spatial proteomic data with AI-based image analysis software, Aivia, we highlight novel molecular circuits disrupted in AD which will help uncover potential biomarkers for early disease detection and therapeutic targeting. This spatially resolved proteomic atlas underscores the power of tissue-based multiplexed proteomics to unravel the cellular choreography of neurodegeneration and opens new avenues for precision neuropathology in AD research.

Disclosures: V. Agrawal: A. Employment/Salary (full or part-time): Leica Microsystems. **R.W. Cho:** A. Employment/Salary (full or part-time): Cell Signaling Technology. **S. Struble:** A. Employment/Salary (full or part-time): Leica Microsystems. **J. Fisher:** A. Employment/Salary (full or part-time): Cell Signaling Technology. **G. Spang:** A. Employment/Salary (full or part-time): Cell Signaling Technology. **S. Sponaugle:** A. Employment/Salary (full or part-time): Cell Signaling Technology. **R. Heil-Chapdelaine:** A. Employment/Salary (full or part-time): Leica Microsystems. **N. Fernandez Diaz Granados:** A. Employment/Salary (full or part-time): Leica Microsystems. **A. Bose:** A. Employment/Salary (full or part-time): Leica Microsystems.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.03/E51

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

Title: Redefining Western Blot Procedures: A Modified Approach for the Reliable Detection of Amyloid-Beta Oligomers (ABOs) in Alzheimer's Research

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Abstract: Neurotoxic amyloid-beta oligomers (AβOs) accumulate in patients with Alzheimer's Disease (AD), inducing the formation of phosphorylated Tau proteins and other AD neuropathology along with the onset of dementia. Being able to fully characterize neurotoxic AβOs and distinguish them from non-toxic oligomers is a critical step in developing targeted therapeutics with the most substantial potential for disease-modifying efficacy. Identifying the size of such oligomers using assays such as Western blots is a vital step in this characterization. However, exposure to the detergent sodium dodecyl sulfate (SDS) in the Western blot procedure may alter the native structure of the oligomeric molecules and can produce misleading results, making characterization difficult. This has led to Western blots being underused when studying AβOs despite the sensitivity and versatility of these blots. This study investigates Western blotting as a technique for studying AβOs by varying the initial concentrations of SDS in the sample and running buffer and evaluating how this can affect the final data obtained in terms of species distribution. Initial results indicate that when running Western blots with different SDS concentrations on synthetic AβO samples, using a reduced concentration of SDS compared to the standard worked more effectively to ensure minimal dissociation of the oligomers while still maintaining the assay's high resolution, showing differing band pattern compared to the classic Western blot on the same samples. When using modified electrophoresis and Western blot conditions, including reduced SDS in the running buffer and complete removal from loading buffer, on wild-type and 5xFAD brain extracts, there was not a significant difference in the displayed signal between the classic Western blot and the modified Western blot results, potentially indicating that mice AβO samples are more "SDS-resistant" than synthetic AβOs. Results were consistent with expectations (low signal in wild-type samples, strong signal in 5xFAD) and were reliably reproducible. We also ran Western blots after 2D gel electrophoresis to substantiate early indications that this approach provides higher resolution than blots of 1D gels. Overall, results indicate that these modifications to the SDS concentrations allow Western blotting to characterize AβOs more accurately while retaining the assay's high overall resolution. These findings can help optimize this widely used technique for AβO research and can be further optimized for other molecules that self-associate.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.04/E52

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

Support: AARG-23-1149996
R01AG058854
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R01AG087513
U01AG068057

RF1NS136995
S10OD032285

Title: Multistudy white matter microstructure associations with amyloid PET

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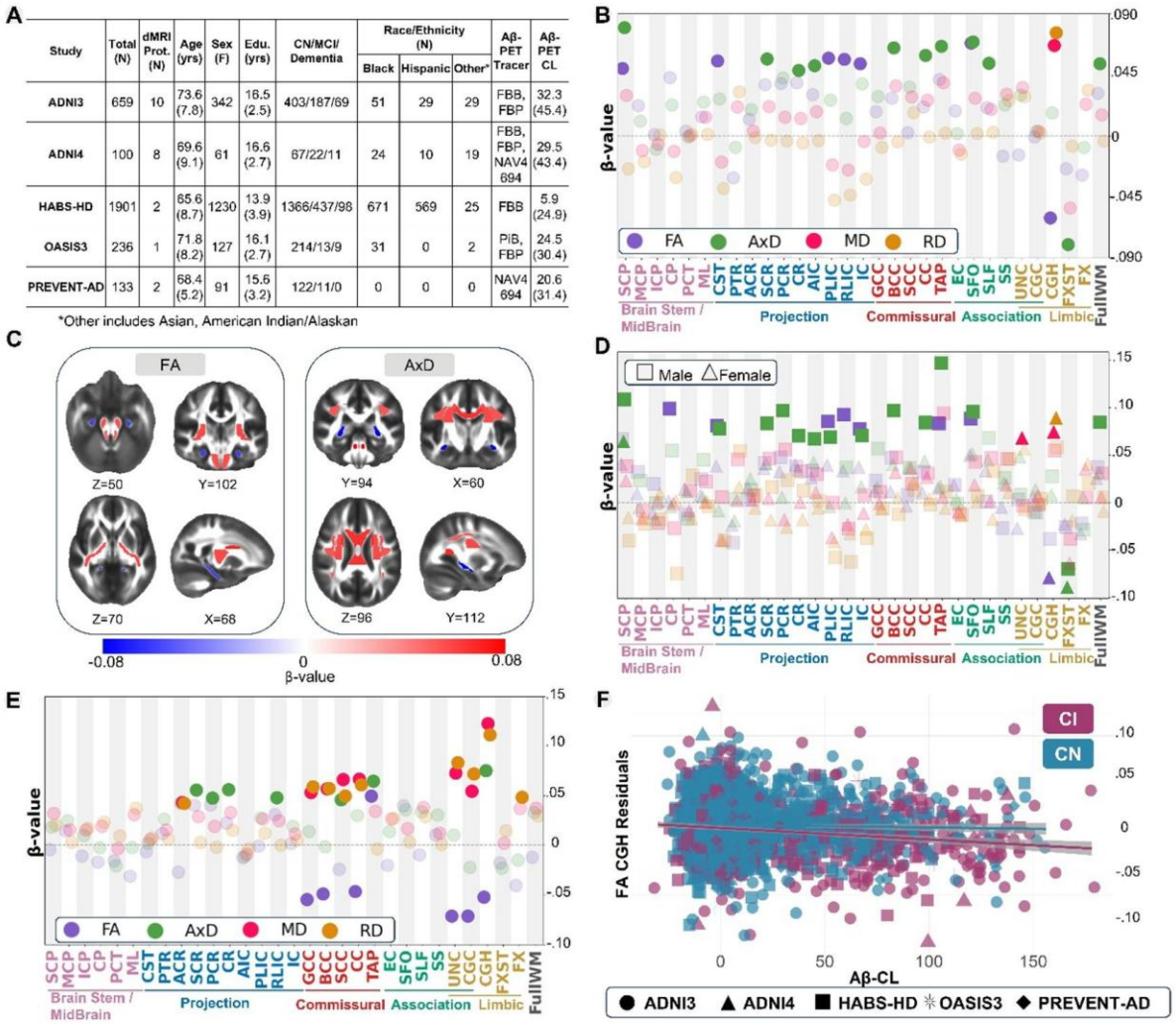
Abstract: Diffusion MRI (dMRI) is sensitive to subtle abnormalities in tissue microstructure and may detect early Alzheimer's disease (AD) neuropathology. While disrupted white matter (WM) microstructure is known to be related to cognitive impairment (CI), its associations with amyloid (Aβ) deposition —the hallmark of AD— remains unclear. We examined WM microstructure using diffusion tensor imaging (DTI) and evaluated its associations with Aβ burden across five AD cohorts.
We analyzed dMRI and Aβ-PET data from 3,029 participants (age = 68.1 ± 9.0 yrs; 38.9% male; 71.7% cognitively normal) from ADNI3/4, HABS-HD, OASIS3, and PREVENT-AD (Fig1A). Mean DTI FA, MD, RD, AxD were extracted from 31 JHU WM atlas regions of interest. Associations between regional dMRI metrics and Aβ-PET centiloids were tested using random-effects linear regressions, adjusting for age, sex, age × sex interactions, race/ethnicity, education, and clinical diagnosis. Study and dMRI protocol were modeled as nested random effects. Follow-up analyses 1) compared effects in males and females, and 2) examined Aβ × CI interactions.
The hippocampal cingulum (CGH) showed the strongest, most consistent associations, with greater Aβ linked to lower FA and higher MD and RD ($P_{FDR}<0.05$; Fig1B). More widespread WM associations were detected between greater Aβ and higher AxD and FA (Fig1C). Males showed widespread associations throughout the WM, while females had fewer associations localized largely to limbic tracts (Fig1D). The effect of Aβ on all 4 dMRI indices was moderated by CI, particularly in the CGH (Fig1E); compared to CN, CI individuals showed steeper increases in diffusivity and decreases in FA with greater Aβ (Fig1F).
Aβ load was linked with alterations in WM microstructure, particularly in limbic tracts. While Aβ effects were often stronger in CI individuals, several tracts —such as association and midbrain pathways— showed significant associations independent of diagnosis. DTI may be sensitive to early WM pathology regardless of CI, though further work is needed to clarify its biological significance.



Study	Total (N)	dMRI Prot. (N)	Age (yrs)	Sex (F)	Edu. (yrs)	CN/MCI/ Dementia	Race/Ethnicity (N)			Aβ-PET Tracer	Aβ-PET CL
							Black	Hispanic	Other*		
ADNI3	659	10	73.6 (7.8)	342	16.5 (2.5)	403/187/69	51	29	29	FBB, FBP	32.3 (45.4)
ADNI4	100	8	69.6 (9.1)	61	16.6 (2.7)	67/22/11	24	10	19	FBB, FBP, NAV4 694	29.5 (43.4)
HABS-HD	1901	2	65.6 (8.7)	1230	13.9 (3.9)	1366/437/98	671	569	25	FBB	5.9 (24.9)
OASIS3	236	1	71.8 (8.2)	127	16.1 (2.7)	214/13/9	31	0	2	PiB, FBP	24.5 (30.4)
PREVENT-AD	133	2	68.4 (5.2)	91	15.6 (3.2)	122/11/0	0	0	0	NAV4 694	20.6 (31.4)

*Other includes Asian, American Indian/Alaskan

Figure 1. A) Summary of demographic and clinical data for ADNI3, ADNI4, HABS-HD, OASIS3, and PREVENT-AD participants. Only those with dMRI and Aβ-PET data acquired within nine months were considered. **B,C)** Effect sizes for regional WM DTI associations with Aβ-CL; significant associations that survive FDR multiple comparisons corrections are opaque (P_{FDR}<0.05). **D)** Effect sizes for regional WM DTI associations with Aβ-CL in Males (N=1178) and Females (N=1851). **E)** Effect sizes for Aβ-by-CI status (i.e., CN vs MCI or Dementia) interaction effects on regional WM DTI metrics. **F)** As an example of Aβ-by-CI trends, a scatter plot of FA residuals in the CGH illustrates that CI individuals showed a steeper decline in anisotropy compared to CN individuals.
KEY: *Aβ-CL: Aβ-PET centiloids; CN: Cognitively Normal; CI: Cognitive Impairment; MCI: Mild Cognitive Impairment; FA: Fractional Anisotropy; MD: Mean Diffusivity; RD: Radial Diffusivity; AxD: Axial Diffusivity; ADNI: Alzheimer's Disease Neuroimaging Initiative; HABS-HD: Health & Aging Brain Study; OASIS: Open Access Series of Imaging Studies; PREVENT-AD: PResymptomatic EValuation of Experimental or Novel Treatments for Alzheimer's Disease; JHU WM Atlas: John Hopkins University ICBM-labels-1mm*

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

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Program #/Poster #: PSTR383.05/E53

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

Support: NIH RF1AG078299
NIH R01AG084624

Title: Cell-class specific miRNAs in normal and Alzheimer's Disease (AD) brains and their relationship to peripheral miRNA candidate AD biomarkers

Authors: *N. UMESH GANESH[1], G. KÜRELI[4], A. KRUNIC[5], F. SANANBENESI[6], T. J. MELLOTT[7], J. K. BLUSZTAJN[2], H. LIN[8], A. FISCHER[9], I. DELALLE[3];
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Abstract: MicroRNAs (miRNAs) are short non-coding RNAs that regulate proteostasis and are emerging as potential prognostic and diagnostic biomarkers for AD. We recently reported that small RNA sequencing of plasma samples collected at baseline from 847 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) revealed miRNAs with changes in the level of expression that correlated with AD diagnosis and helped predict the conversion from early mild cognitive impairment (MCI) to AD [PMID 39291752]. Moreover, sveral miRNA levels were significantly associated with A/T/N positivity [PMID 39291737]. To validate candidate miRNA biomarkers and explore the relationship between brain and plasma miRNA signatures, small RNA sequencing was performed on 3-5 longitudinal plasma samples, before and after AD diagnosis, and on cerebral cortical cells obtained from postmortem brains of 21 individuals from the Framingham Heart Study (FHS). They comprised 12 "true controls", i.e. individuals with no cognitive impairment whose brains revealed no pathological changes diagnostic of any neurodegenerative diseases (NDs), and 11 "pure" AD cases, Braak stages 3 - 6, without other NDs pathologies present. Small RNAs including miRNAs were extracted from ~ 4,000 cells (per cell-type), Laser-Capture-Microdissected (LCMed) individually from cryosections of prefrontal and hippocampal cortices to allow for correlative FHS phenotypic analyses and the validation of plasma miRNA signatures associated with MCI and AD diagnosis in FHS and ADNI. LCM-ed neurons, astrocytes, and microglia had cell-class specific miRNA signatures and several miRNAs showed AD diagnosis-associated expression alteration. Our study provides a comprehensive characterization of miRNA profiles during AD progression and offers insights into pathogenetic mechanisms critical for the development of disease-modifying therapies.

Disclosures: N. Umesh Ganesh: None. **G. Küreli:** None. **A. Krunic:** None. **F. Sananbenesi:** None. **T.J. Mellott:** None. **J.K. Blusztajn:** None. **H. Lin:** None. **A. Fischer:** None. **I. Delalle:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

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

Support: National Research Foundation of Korea(NRF) funded by the Korean government(MSIT), Bio & Medical Technology Development Program (RS-2024-00441331)
Chang Won MB2302.

Title: Novel microRNA biomarkers that include functions related to synapses plasticity and neuroinflammation in human blood and convergence diagnostic medical device including cartridge for early Alzheimer's disease

Authors: K. CHOE[1,2], M. KANG[1], J. PARK[1], S. MA[3], ***M. KIM**[1];
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Abstract: Alzheimer's disease (AD), an intractable disorder responsible for about 80 % of dementia, is detectable by MRI or PET only after it reaches a moderate or severe stage; hence blood-based biomarkers are urgently needed. Here, we discovered five novel 18-22 nt microRNAs (miRNAs) for early AD diagnosis. EDTA blood from cognitively normal, mild cognitive impairment (MCI) and AD subjects was processed to plasma, stored at -70 °C. Total RNA was extracted. Small-RNA Libraries were sequenced on a NovaSeq platform. Adapter, quality and length trimming preceded miRDeep2* analysis. A new miRNA having a miRDeep2* score of -10 to 10 is derived. Among them, we chose miRDeep2* score ≥ 1, RNAfold of "yes" and a number of feature reads ≥ 10 are selected. miRDB target-prediction scores were then applied to rank candidate targets, after which we filtered for high-scoring, Alzheimer's disease-relevant genes. One of the newly identified microRNAs targets MAP1A, a protein crucial for microtubule assembly and the regulation of synaptic plasticity. A second novel microRNA is predicted to regulate TANC2, which is indispensable for dendrite formation during synaptic remodeling. A third novel microRNA targets both MAP1A and NR1D1, the latter known for repressing inflammatory genes and promoting amyloid-β clearance. A fourth novel microRNA is predicted to down-regulate SHANK2, a scaffold that maintains synaptic structural stability and plasticity, as well as FOXO1, a transcription factor that supports neuronal survival, energy homeostasis, and limits amyloid-β accumulation. Finally, the fifth novel microRNA targets SNAP-25, a core component that participates in multiple steps of neurotransmitter release. SH-SY5Y neuroblastoma cells were transfected with a five novel miRNA mimic and miRNA inhibitor; western blotting and immunofluorescence showed that overexpression of miRNA markedly reduced target protein, whereas inhibition increased it. In addition, target sequencing to analyze the expression level of novel miRNAs in plasma of patients classified as normal, MCI, and AD confirmed the significant difference in the expression level of miRNAs in each group. Thus, we have isolated a circulating five novel miRNAs that exhibits high specificity and sensitivity, modulates an AD-relevant target, and discriminates clinical stages, underscoring its potential as a minimally invasive biomarker for early AD diagnosis. And It is also possible to

develop a convergence diagnostic medical device including a new miRNA detectable dedicated PCR cartridge.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

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Program #/Poster #: PSTR383.07/E55

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

Support: NIH RF1 AG078299
NIH R01 AG084624

Title: The Alzheimer's disease (AD)-associated biomarker, miR-223-3p, is neurotrophic and protects against AD-related pathophysiologic pathways

Authors: *A. KRUNIC[1], G. KÜRELI[1], N. UMESH GANESH[1], A. FISCHER[2], I. DELALLE[1], T. J. MELLOTT[1], J. K. BLUSZTAJN[1];
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Abstract: MicroRNAs (miRNA) are promising biomarkers and potential targets of interventional strategies for the management of AD. Previous work has shown that the levels of miR-223-3p are increased in plasma and brains of patients with AD as compared to normal individuals and that its levels can be used to predict conversion from mild cognitive impairment to dementia. However, the role of miR-223-3p in the brain and in the context of AD pathophysiology is understudied. To investigate the functions of miR-223-3p *in vitro*, we transfected human iPSC-derived neurons (iNeurons) with miR-223-3p mimics and conducted RNA-sequencing and immunocytochemistry. miR-223-3p mimic-treated iNeurons were characterized by the downregulation of expression of its predicted mRNA targets together with an upregulation of expression of neuron structural genes and protective amyloid binding protein A2M. Furthermore, we observed downregulation of the IL6ST(gp130)/JAK/STAT signaling pathway and lipid metabolism-related genes. On a morphological level, miR-223-3p enhanced neurite outgrowth during neuronal maturation *in vitro*, suggesting a neurotrophic role. To assess its function *in vivo*, we injected wild type or *App*$^{NL-G-F}$ AD model mice intracerebroventricularly with miR-223-3p mimic, antagonist, or scrambled control, followed by behavioral assessment by Barnes maze and contextual fear conditioning. Inhibition of miR-223-3p impaired fearful learning in females, but not in males, indicating a sex-specific role of this miR in cognition and suggesting that overexppression of miR-223-3p could potentially ameliorate behavioral deficits in the *App*$^{NL-G-F}$ model of AD. Overall, our findings highlight the neurotrophic functions of miR-223-3p and support its potential as novel therapeutic agent for AD.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

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

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TTUHSC EP MTM Startup Funds

Title: Mirna-501-3p and mirna-502-3p: a promising biomarker panel for alzheimer's disease

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Abstract: INTRODUCTION: Alzheimer's disease (AD) lacks a less invasive and early detectable biomarker. Here, we investigated the biomarker potential of miR-501-3p and miR-502-3p using different AD sources. **METHODS:** MiR-501-3p and miR-502-3p expressions were evaluated in AD CSF exosomes, serum exosomes, familial and sporadic AD fibroblasts, and B-lymphocytes by qRT-PCR analysis. Further, miR-501-3p and miR-502-3p expressions were analyzed in APP, Tau cells media exosomes, and in different brain cell types. **RESULTS:** MiR-501-3p and miR-502-3p expressions were significantly upregulated in AD CSF exosomes relative to controls. MiRNA levels were high in accordance with amyloid plaque and NFT density in multiple brain regions. Similarly, both miRNAs were elevated in AD and MCI serum exosomes compared to controls. MiR-502-3p expression was high in fAD and sAD B-lymphocytes. MiR-501-3p and miR-502-3p expression were elevated intracellularly and secreted extracellularly in response to APP and Tau pathology. Finally, neurons and astrocytes displayed high expression of these miRNAs. **DISCUSSION:** These results suggest that miR-501-3p and miR-502-3p could be promising biomarkers for AD.

Disclosures: D. Devara: None. **B. Sharma:** A. Employment/Salary (full or part-time): Texas Tech University Health Sciences Center El Paso. **G. Goyal:** A. Employment/Salary (full or part-time): Penn State College of Medicine. **D. Rodarte:** A. Employment/Salary (full or part-time):

Texas Tech University Health Sciences Center El Paso. **A. Kulkarni:** None. **N. Tinu:** None. **S. Kumar:** A. Employment/Salary (full or part-time): Texas Tech University Health Sciences Center El Paso.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.09/E57

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

Support: National Research Foundation of Korea(NRF) funded by the Korean government(MSIT), Bio & Medical Technology Development Program (RS-2024-00441331)
Division of Life Science and Applied Life Science BK21 FOUR(BrainKorea21Four)

Title: Development of a medical device for in vitro diagnosis of mild cognitive impairment (MCI) and Alzheimer's disease using novel microRNA biomarkers that include functions related to synapses and amyloid-β in human blood

Authors: *M. KANG[1], J. PARK[1], K. CHOE[1,2], H. PARK[2,3], H. KANG[4], M. KIM[1];
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Abstract: Alzheimer’s disease (AD), an intractable disorder responsible for about 80 % of dementia, is detectable by MRI or PET only after it reaches a moderate or severe stage; hence blood-based biomarkers are urgently needed. Here, we discovered five novel 18-22 nt microRNAs (miRNAs) for early AD diagnosis. EDTA blood from cognitively normal, mild cognitive impairment (MCI) and AD subjects was processed to plasma, stored at -70 °C. Total RNA was extracted. Small-RNA Libraries were sequenced on a NovaSeq platform. Adapter, quality and length trimming preceded miRDeep2* analysis. A new miRNA having a miRDeep2* score of -10 to 10 is derived. Among them, we chose miRDeep2* score ≥ 1, RNAfold of "yes" and a number of feature reads ≥ 10 are selected. miRDB target-prediction scores were then applied to rank candidate targets, after which we filtered for high-scoring, Alzheimer’s disease-relevant genes. We identified a first novel microRNA that targets SYNJ1, related to synaptic-vesicle recycling; SNAP91, essential for vesicle transport and synaptic transmission; and HOOK3, an adaptor involved in neuronal endosomal trafficking. A second novel microRNA is predicted to regulate BDNF, a core mediator of synaptic transmission and plasticity; NEFL, which maintains axonal structural stability and is linked to axonal injury; and NRG3, a factor critical for glial growth, differentiation. A third novel microRNA also targets HOOK3,

connecting it to both endosomal transport and the generation of amyloid-β. A fourth novel microRNA is that target MAP1A, a protein vital for microtubule assembly and the modulation of synaptic plasticity. Finally, a fifth novel microRNA targets SHANK1, a postsynaptic-density scaffold that preserves the structural and functional integrity of dendritic spines and excitatory synapses. SH-SY5Y neuroblastoma cells were transfected with a five novel miRNA mimic and miRNA inhibitor; western blotting and immunofluorescence showed that overexpression of miRNA markedly reduced target protein, whereas inhibition increased it. In addition, target sequencing to analyze the expression level of novel miRNAs in plasma of patients classified as normal, MCI, and AD confirmed the significant difference in the expression level of miRNAs in each group. Thus, we have isolated a circulating five novel miRNAs that exhibits high specificity and sensitivity, modulates an AD-relevant target, and discriminates clinical stages, underscoring its potential as a minimally invasive biomarker for early AD diagnosis. And It is also possible to develop a convergence diagnostic medical device including a new miRNA detectable dedicated PCR cartridge.

Disclosures: M. Kang: None. **J. Park:** None. **K. Choe:** None. **H. Park:** None. **H. Kang:** None. **M. Kim:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.10/E58

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

Support: NIH/NIA AG077700

Title: Evaluation of drug effects on the binding of [^{125}I]IPPI and [^{18}F]MK-6240 to human tau in postmortem brains of human Alzheimer's disease and Down syndrome

Authors: F. KARIM, A. P. BIJU, D. M. SCHAFER, S. A. SISON, C. LIANG, *J. MUKHERJEE;
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Abstract: Objective: The accumulation of tau tangles is a prominent biomarker that characterize Alzheimer’s disease (AD) and Down Syndrome (DS). Continuous developments of PET tracers aim to accurately assess the abundance of biomarkers such as tau in different brain regions to elucidate the pathophysiology of DSAD and AD. This study aims to assess binding of [^{125}I]IPPI and [^{18}F]MK-6240, imaging probes for pathological, aggregated tau, in AD and DSAD human frontal cortex (FCX) and temporal cortex (TCX) postmortem brain slices and measure effects of drugs known to bind to tau, monoamine oxidase A (MAO-A) and dual specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A).
Methods: In this comparative study, quantitative [^{125}I]IPPI and [^{18}F]MK-6240 binding to tau in postmortem human brain slices of FCX and TCX regions were evaluated for all AD (n=5; 3 male

and 2 female), DSAD (n=5; 2 male and 3 female), and cognitively normal (CN) (n=5; 2 male and 3 female) subjects. Adjacent brain slices were immunostained for tau and images were analyzed by QuPath. The autoradiographic images of [^{125}I]IPPI and [^{18}F]MK-6240 binding to adjacent brain slices were measured in OptiQuant.
Results: There was significantly more [^{125}I]IPPI and [^{18}F]MK-6240 binding in AD and DSAD gray matter (GM) than CN subjects, which positively correlated with the percent of anti-tau immunostaining. The GM/white matter ratios for DSAD ([^{125}I]IPPI=4.1; [^{18}F]MK-6240=2.2) and AD ([^{125}I]IPPI=3.6; [^{18}F]MK-6240=1.9) were significantly greater than CN subjects ([^{125}I]IPPI=1.2; [^{18}F]MK-6240=1.4). In vitro drug inhibition studies revealed that with increasing harmine concentrations, [^{125}I]IPPI binding and [^{18}F]MK-6240 binding were significantly inhibited. Measured affinities of harmine for tau were: AD IC_{50}=135 nM; DSAD IC_{50}=115 nM (using [^{125}I]IPPI) and AD IC_{50}=111 nM; DSAD IC_{50}=129 nM (using [^{18}F]MK-6240). The selective DYRK1A inhibitor KuFal194 and selective MAO-A inhibitor clorgyline minimally inhibited [^{125}I]IPPI and [^{18}F]MK-6240 binding, suggesting the two imaging probes selectively bound to tau in both AD and DSAD.
Conclusion: In addition to the established findings in AD, [^{18}F]MK-6240 and [^{125}I]IPPI show similar binding to tau in DSAD subjects in FCX and TCX regions. Harmine is a nonselective drug, with an affinity profile of MAO-A (5 nM) > DYRK1A (70 nM) > Tau (111-135 nM, measured in this work) and would be considered unsuitable for drug challenge studies due to its multi-target profile. This study demonstrates promise of [^{125}I]IPPI and [^{18}F]MK-6240 as viable and selective tau radiotracers to assist in the diagnostic imaging of AD and DSAD in humans.

Disclosures: F. Karim: None. **A.P. Biju:** None. **D.M. Schafer:** None. **S.A. Sison:** None. **C. Liang:** None. **J. Mukherjee:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.11/F1

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

Support: JSPS KAKENHI Grant 24KJ1819

Title: Development of Classifier with Universality for Diagnosis of Mild Cognitive Impairment Using Electroencephalography

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Abstract: Proper diagnosis of mild cognitive impairment (MCI) is one of the important challenges in the early detection of dementia. Many studies have been conducted to construct classifiers to predict MCI with high accuracy, but the results might depend on the subject groups. In this study, the universality of classifier was evaluated based on whether classifier that had the same features across all subject groups but were learned for each group showed high accuracy across all groups. The subject groups consisted of 7 MCI patients and 10 healthy controls (Japanese group), and 29 MCI patients and 32 healthy controls (Iranian group). Electroencephalography (EEG) data of the common 16 regions were separated into 6 frequency bands. Classifiers were constructed for each subject group and each frequency band. Power spectrum and functional connectivity were calculated as features for classifier. Standardized and exponentially transformed values of these features were also adapted. In addition, graph metrics based on local, global connectivity, and centrality were calculated by functional connectivity. Construction of classifiers was as follows. First, features were selected. In one subject group, all subjects were excluded one by one to create datasets. Recursive Feature Elimination (RFE) were applied to each dataset to select features. Features selected in more than 75% of the entire dataset was used for classifier. With selected features, accuracy of classifier was calculated by Leave-One-Out Cross Validation (LOOCV) for each subject group. The same features were used in the two groups, but the weights of the features were different for each group. We selected the classifier that showed the highest accuracy in both groups. As a result, the classifier with the highest accuracy was confirmed for lower alpha band. This classifier consisted of 5 features selected from standardized and exponentially transformed power spectrum, functional connectivity, and graph metrics by standardized and exponentially transformed functional connectivity. Accuracies by Gradient Boosting were 76% in Japanese group and 93% in Iranian group. Features of this classifiers mainly consisted of the frontal and occipital lobes. Important features were functional connectivity of the occipital lobe in Iranian group, and that of the frontal and occipital lobes in Japanese group. The results of the two subject groups suggest that features of the frontal and occipital lobes may be associated with cognitive dysfunction in MCI patients. In addition, these features may be important for construction of a universal classifier and for diagnosis.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.12/F2

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

Support: Davis APP grant from the Shelby Collum Davis Fund
NIH Grant P01AG015379
NIH Grant RF1AG006173

Title: Monoclonal antibody 71A1 detects low MW oAβ more sensitively than lecanemab in human biofluids: a comparison in CSF and plasma

Authors: *Y. XU[1], T. YANG[2], B. OSTASZEWSKI[2], A. K. ANDERSON[3], H.-S. YANG[4], D. WALSH[5], T. L. LAWTON[7], D. J. SELKOE[6];
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Abstract: Mounting evidence suggests small, cytotoxic, aqueously diffusible Amyloid-beta oligomers (oAß) play a pivotal role in initiating the pathogenic cascade in Alzheimer's disease (AD), including microgliosis, astrogliosis, and tau+ neuritic dystrophy. Accurate quantification of bioactive oAß species in brain and human biofluids could be valuable to understand disease progression in AD and related dementias (ADRD), but very few validated assays for this hydrophobic analyte exist. We evaluated the ability of two monoclonal antibodies with different oligomer preferences to detect the oAß species in AD brain tissue, cerebrospinal fluid (CSF), and plasma. The antibody 71A1 preferentially binds smaller oAß aggregates, while lecanemab targets protofibrils. Both assays are performed on the SMCxPRO platform, using 71A1 or lecanemab as the capture antibody and N-terminal specific Aβ antibody 3D6 as the detector. Both assays show similar dose-dependent responses to synthetic Aβ-Derived Diffusible Ligands (ADDLs, aggregated Aß42) with picogram per ml sensitivities. 71A1 specifically showed considerably higher reactivity to smaller crosslinked dimers of synthetic Aß (Aß(S26C)$_2$) than lecanemab; this dimer signal of 71A1 was unchanged even when excess lecanemab was spiked into solution, supporting the antibodies' differential preference for oligomer size. For each assay, we observed significantly increased levels of oAβ in Alzheimer than in control brain extracts. In CSF of patients clinically diagnosed with AD, we observed strong positive correlations (r) between levels of 71A1-reactive oAß and total tau (r = 0.66) and phosphorylated tau-181 (r = 0.68) measured by commercial Mayo Clinic ADEVL assays. These patients also showed significantly higher oAβ levels than control individuals on average. In contrast, the lecanemab readouts on the same CSF samples were below the quantification limit of that assay and showed no correlation with established AD biomarkers, suggesting a limited presence of lecanemab-reactive species in human CSF. We then assayed plasmas from the MGH Down Syndrome Cohort (age 0-60) and observed a strong inverse correlation between age and 71A1-reactive oAß levels. In contrast, no such trends were observed with lecanemab. These results together suggest that while lecanemab has proven to be a clinically efficacious immunotherapeutic antibody, its target may be underrepresented in biofluids. Conversely, 71A1 robustly detects AD biomarker-relevant oAβ species in CSF and plasma, supporting its potential as a novel biomarker to monitor progression and treatment response in AD.

Disclosures: Y. Xu: None. **T. Yang:** None. **B. Ostaszewski:** None. **A.K. Anderson:** None. **H. Yang:** None. **D. Walsh:** None. **T.L. Lawton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); T.L.L. is the technical founder and employee of Abyssinia Biologics, LLC. **D.J. Selkoe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DJS is a director of Prothena Biosciences. F. Consulting Fees (e.g., advisory boards); DJS is a consultant to Eisai and Roche.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.13/F3

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

Support: Posdoctoral fellowship SECIHTI Granted to M.C.S.-L.
SECIHTI Grant-319578

Title: Evaluation of biomarkers of Mild Cognitive Impairment in olfactory neuroepithelial cells and plasma from Mexican individuals

Authors: *M. SILVA-LUCERO[1,2], O. LORA-MARIN[2], J. PADILLA-MENDOZA[2], M. CARDENAS-AGUAYO[2];
[1]Physiol., UNAM, Ciudad de México, Mexico; [2]Physiology, Lab. of Cell. Reprogramming, Sch. of Medicine, Natl. Autonomous Univ. of Mexico (UNAM), Ciudad de Mexico, Mexico

Abstract: Introduction: Alzheimer's disease (AD), the leading cause of dementia, is characterized by a prolonged presymptomatic phase marked by progressive neurobiological changes. Early identification of individuals with Mild Cognitive Impairment (MCI), a prodromal stage of AD, is essential for developing effective interventions, especially in the current absence of disease-modifying therapies. Although PET imaging has proven useful in detecting AD biomarkers, its high cost limits its widespread clinical use. In response, our research group proposes an alternative approach that is more accessible, based on the detection of biomarkers in olfactory neuroepithelial cells and peripheral plasma. **Methodology:** We recruited a cohort of older adults with clinical signs of MCI and a control group of cognitively healthy individuals. All participants underwent cognitive screening using the Montreal Cognitive Assessment (MoCA), a comprehensive medical interview, and blood sampling for plasma extraction. In addition, participants completed an olfactory test and a non-invasive nasal scraping procedure to isolate and characterize precursor cells from the olfactory epithelium (hONE). Once cell cultures were established, several biomarkers were assessed using Western blot analysis. **Results:** We obtained clinical, cognitive, and molecular data from 60 Mexican participants. Cognitive status was assessed through MoCA and clinical history. Plasma levels of b-amyloid (AB) were quantified from blood samples, but no significant differences were observed. Cultured hONE cells were analyzed for several biomarkers using Western blot, including amyloid precursor protein (APP), total tau (T-tau), and phosphorylated tau at threonine 217 (p-tau217), and only a trend in an increase in phosphor-tau was observed. Additional biomarkers identified through bioinformatic analysis were also evaluated, including the calcineurin subunit (a Ca^{2+}/calmodulin-regulated serine/threonine phosphatase), B-synuclein (associated with synaptic degeneration), and α-synuclein. **Conclusion:** The identification of altered genes and proteins in peripheral cells may facilitate the development of early, cost-effective biomarkers for the diagnosis of Alzheimer's disease.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.14/F4

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

Support: the National Natural Science Foundation of China (Grant No. 82101492)

Title: Cognitive reserve characteristics and neurophysiological mechanisms based on olfactory working memory capacity task

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Abstract: The concept of cognitive reserve originated from the observation of human neurodegenerative diseases and cognitive decline, aiming to explain why different individuals show different levels of cognitive function under similar brain pathological burdens. Cognitive reserve reflects the flexibility of the brain to use existing neural networks and compensatory mechanisms, so that it can still maintain good cognitive performance with pathological changes. The reserve capacity slows down the decline of cognitive function by improving information processing efficiency and nervous system adaptability and is considered to be an important protective factor for preventing and delaying cognitive decline. Although there are a lot of studies on human cognitive reserve, there is still a gap in establishing a systematic and standardized cognitive reserve measurement method in experimental animals such as mice. We performed cognitive reserve tests through olfactory working memory capacity tasks and used the average working memory capacity of normal control mice as the normal baseline level of cognitive reserve tests. If the working memory capacity level of Alzheimer's disease model mice was greater than or equal to the normal baseline level, they were mice with high cognitive reserve levels. Conversely, if the working memory capacity level of Alzheimer's disease model mice was less than the normal baseline level, they were mice with low cognitive reserve levels. Our research results showed that the olfactory working memory capacity task can be used to screen the cognitive reserve of Alzheimer's disease model mice. The working memory capacity of high cognitive reserve 5×FAD mice was significantly higher than that of low cognitive reserve 5×FAD mice ($p < 0.05$). In addition, we further explored the role of prefrontal neuron discharge activity in the cognitive reserve process. We found that compared with low cognitive reserve 5×FAD mice, high cognitive reserve 5×FAD mice had increased neuronal discharge in the working memory maintenance stage during the olfactory working memory capacity task ($p < 0.05$). The encoding and regulation of working memory capacity involve higher-order centers such as the prefrontal cortex, and pathology and dysfunction of the prefrontal cortex occur in the early stages of Alzheimer's disease, indicating that the prefrontal cortex may play an important

role in cognitive reserve and will provide a theoretical basis for the early prevention and intervention of neurodegenerative diseases.

Disclosures: G. Huang: None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.15/Web Only

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

Support: University of Guanajuato 180-2025
Conahcyt ApoyosLNC-2023

Title: Thinning of the retinal ganglion cell layers is correlated with global cognitive impairment, delayed recall memory decline and body mass index in women with sporadic Alzheimers disease.

Authors: *M. SOLIS-ORTIZ[1], N. HERNÁNDEZ SEBASTIÁN[4], M. GUERRERO VILLALPANDO[1], C. MARIN ARANDA[2], M. CARDONA ALVARADO[3];
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Abstract: Alzheimers disease AD is a neurodegenerative disease that leads to severe cognitive impairment, particularly of episodic memory. Retinal ganglion cells are an excellent biomarker for detecting AD, given their connection to the brain and obesity has been linked to cognitive decline. However, the impact of retinal ganglion cell layer thickness on cognitive decline, episodic memory and anthropometric measurements in AD is not well known. The objective of the present study was to investigate the association of retinal ganglion cell layer thickness with delayed recall memory, global cognitive performance, and body mass index in women with sporadic AD. Seventy seven elderly women between 60 and 80 years of age participated in the study. The elderly women were 28 clinically diagnosed with mild cognitive impairment, 32 with moderate cognitive impairment and 17 with severe cognitive impairment in the progression of AD, in accordance with The Montreal Mental Cognitive Assessment test scores. This test was also used to assess global cognitive performance and the delayed recall memory. The body mass index was also obtained. An Ocular Coherence Tomography was performed on the participants to measure the minimum and average thickness of the retinal ganglion cell layers. Elderly women with severe cognitive impairment showed thinning of the ganglion cell layers in both eyes, lower scores on the MoCa test, delayed recall memory impairment and overweight. This thinning was positively correlated with global cognitive impairment and lower scores on delayed recall memory and was negatively correlated with body mass index. These findings suggest that retinal ganglion cell thinning may influence impaired cognitive function and the inability to

recall past events. Furthermore, the inflammation caused by excess weight may be damaging retinal ganglion cells in women with sporadic AD.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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

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The Hertz Innovation Fund

Title: Tauopathy-laden retinal ganglion cells undergo granulovacuolar degeneration-associated necroptosis in Alzheimer's and non-Alzheimer's dementia

Authors: *M. DAVIS[1], Y. KORONYO[1], E. ROBINSON[1], E. SALOBRAR-GARCIA[2], A. RENTSENDORJ[1], B. GAIRE[1], R. KAYED[3], A. SADUN[4], L. SCHNEIDER[5], K. BLACK[1], D.-T. FUCHS[1], M. KORONYO-HAMAOUI[1];
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Abstract: Studies haved demonstrated a loss of retinal ganglion cells (RGCs) in Alzheimer's disease (AD) and non-AD dementia patients, potentially contributing to visual dysfunctions. We reported elevated pathogenic-tau isoforms, hyperphosphorylated tau at serine 396 (pS396-tau) and tau oligomers (oligo-tau), in the retinas of patients with mild cognitive impairment (MCI, due-to-AD) and AD dementia compared to cognitively normal (CN) subjects. We show that immunohistochemical staining identified granulovacuolar degeneration bodies (GVD) and granulovacuolar-mediated necroptosis (GVD-necroptosis) positive RGCs in individuals with MCI and AD. Here we explore the link of tauopathy-laden RGCs to GVD-necroptotic cells in tMCI, AD-dementia, and non-AD dementia. Retinal cross-sections from 25 patients with MCI or AD-dementia, 8 non-AD dementia and 10 age- and sex-matched CN controls were analyzed. Immunofluorescence staining was conducted, using the RGC marker ribonucleic acid binding

protein with multiple splicing (RBPMS) in conjunction with pathogenic tau-isoforms, and markers of apoptosis, GVD, and GVD-necroptosis markers, charged multivesicular body protein 2B (CHPM2B) and phosphorylated mixed lineage kinase domain-like pseudokinase (pMLKL), respectively. Stereological quantifications and analyses of RGC morphology were performed. Findings were correlated with brain pathology and cognitive status. We show pS396^{+}-tau and T22^{+} oligo-tau inclusions within RBPMS^{+} RGCs, alongside a 46-56% reduction in RBPMS^{+}-RGCs of MCI and AD retinas. RGC loss is accompanied by morphological and molecular alterations: soma hypertrophy (10-50% enlargement), nuclear displacement, apoptosis (30-50% increase), and an increased expression of CHPM2B^{+} and pMLKL^{+} markers in MCI and AD, compared to CN. PS396-tau^{+}-RGC and oligo-tau^{+}-RGC counts were 2.1-3.5-fold higher in MCI and AD retinas compared to CN. In non-AD dementia, we showed a significant decrease of paired helical filament-tau and T22 oligo-tau compared to AD. Tau-laden RGCs intercorrelated ($r= 0.85$, $P<0.0001$), and were associated with RGC reduction ($r= -0.40$-(-0.64), $P<0.05$-0.01). Their abundance is linked to advanced Braak stage (V-VI) and cognitive decline. Our findings suggest that abnormal tau-isoforms within RBPMS^{+} RGCs in MCI and AD patients contribute to early RGC loss and correlate with disease severity. This link involves apoptotic and GVD-necroptotic cell death pathways. The results should be validated in larger cohorts to develop RGC-tauopathy as a noninvasive biomarker for early AD detection and monitoring.

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Poster

PSTR383: Alzheimer's Disease: Biomarkers

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.17/F6

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

Support: Davis Alzheimer Prevention Program

Title: Nt1-tau in plasma shows predictive potential over brain-derived tau for alzheimer's disease

Authors: *A. K. ANDERSON[1], B. OSTASZEWSKI[2], T. YANG[2], Y. XU[3], A. STERN[2], D. J. SELKOE[4];
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Abstract: Tau's role in axonal transport is disrupted in the presence of stress, where its phosphorylation alters conformation and triggers its cleavage and release into the intracellular space. As a key biomarker for Alzheimer's disease (AD), tau is measured via PET imaging and

cerebrospinal fluid, however, blood-based tests are more economical and less invasive. Several tau biomarkers show promise in AD diagnostics. Our previous work on subjects with familial AD or Down syndrome has shown an N-terminal tau fragment, NT1, as a promising predictor of AD, correlating with age and disease. Here, we explore an NT1 plasma immunoassay in monitoring aspects of AD. Human subjects were recruited for 6 studies on AD risk, including 3 Low risk of AD (L) and 3 High risk of AD (H) based on age, clinical evaluation, imaging, or Mayo Clinic assays: L1 (n=143), L2 (n=106), L3 (n=2847), H1 (n=361), H2 (n=361), H3 (n=299). ELISA immunoassays were run on AD biomarkers in CSF and plasma, including NT1, pTau217, brain-derived tau (BDT), and oligomeric Aβ (oAβ), or measured using Mayo Clinic CSF ADEVL tests for pTau181 and Aβ42. Quantitative analyses assessed correlation, analytical performance, and clinical performance. NT1 showed strong consistency across two commercial platforms (HD1 vs. the updated HDX) and retested cohorts over two years. An unpaired t-test showed High risk AD subjects had significantly higher plasma NT1 levels (mean=7.5pg/ml, SD=11.5) compared to Low risk AD (mean=4.5pg/ml; SD= 13.2). In hospital cohorts (L1 and L2), NT1 showed a strong negative correlation with cognitive function. In CSF and plasma, both NT1 and BDT strongly correlated. ADEVL Aβ42 in CSF had similar weak negative correlations to plasma pTau217, plasma NT1, and plasma BDT. However, against CSF oAβ levels, both CSF BDT and NT1 had comparable moderate correlations. In CSF, ADEVL pTau181 and total Tau had comparable strong correlations against NT1 and BDT, but in plasma, NT1 outperformed BDT. Analytical performance showed plasma NT1 (AUC=0.676) performed similarly to BDT (AUC=0.787). Multiple logistic regression prediction models using CSF ADEVL Aβ42/pTau181 and either plasma NT1 or BDT to predict clinical AD diagnosis showed that NT1 had a higher percent correct (95.4%) than BDT (87.5%). In conclusion, NT1 and BDT are generally comparable biomarkers; however, in plasma, NT1 may outperform BDT in predictive power. NT1 could replace total tau or neurofilament light (NfL) as the preferred neurodegenerative marker of the ATN AD diagnostic framework (A = amyloid, T = tau, N = neurodegeneration). More work is needed to confirm clinical relevance by validating these tau biomarkers against "big tau", NfL, and total tau.

Disclosures: A.K. Anderson: None. **B. Ostaszewski:** None. **T. Yang:** None. **Y. Xu:** None. **A. Stern:** None. **D.J. Selkoe:** 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.; D.J.S. is a director and consultant to Prothena Biosciences..

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.18/F7

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

Title: Enhanced characterization of phospho-tau residues using highly specific recombinant antibodies

Authors: *A. RAHMAN-ENYART[1], Y. LU[2], L. LIAN[2], D. SHANKAR[1];
[1]Proteintech Group, Rosemont, IL; [2]Proteintech Group, Wuhan, China

Abstract: Alzheimer'sdisease (AD) is a progressive neurodegenerative disorder and the leading causeof dementia in older adults. One of the pathological hallmarks of AD areneurofibrillary tangles comprised of hyperphosphorylated aggregates of themicrotubule associated protein tau. Over 85 potential phosphorylation sitesspan the tau protein, and the accurate detection and mapping of phosphorylatedresidues are essential for understanding disease mechanisms and developingtargeted therapeutics for AD. However, the generation of antibodies fordetection of posttranslational modifications, such as phosphorylation, can bechallenging. Here we present an enhanced approach for phospho-tau detection inimmunoassays using recombinant monoclonal antibodies targeting key tauphosphorylation sites, including pSer202/pThr205, pSer404, and pThr231.Leveraging the advantages of recombinant technology, we were able to achievestable and high expression of recombinant monoclonal antibodies against uniquepTau sites by cloning antibody heavy and light chain sequences derived fromantigen-specific B cells into high-yield expression vectors followed by theirintroduction into suitable mammalian expression systems. These antibodies wereevaluated in multiple immunoassay applications including western blot, ELISA,and flow cytometry, and demonstrated enhanced specificity and high targetaffinity for their respective pTau sites. Furthermore, using peptide scanning,microbial display, and AI modeling, we mapped the exact binding sites of eachantibody on the 3D structure of the tau protein, allowing for further insighton the antibody-pTau interaction. Overall, our findings highlight theadvantages of recombinant antibodies, over traditional hybridoma derivedclones, as robust tools for analyzing tau phosphorylation in both physiologicaland pathological contexts.

Disclosures: A. Rahman-Enyart: A. Employment/Salary (full or part-time): Proteintech. **Y. Lu:** A. Employment/Salary (full or part-time): Proteintech. **L. Lian:** A. Employment/Salary (full or part-time): Proteintech. **D. Shankar:** A. Employment/Salary (full or part-time): Proteintech.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.19/F8

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

Title: Ultra-sensitive differentiation of p-tau 217 in mci and dementia patients: comparing amyloid pet negative and positive results

Authors: *C. WORSFOLD, C. FAN;
Spear Bio, Woburn, MA

Abstract: Method: Successive Proximity Extension Amplification Reactions (SPEAR) utilizes a unique two-factor authentication mechanism for attomolar detectability from 1 µL of diluted sample, employing a 3-step, wash-free workflow with readout on standard qPCR instruments.

The SPEAR UltraDetect™ pTau 217 immunoassay was tested with 101 donors diagnosed with MCI (67) or dementia (34) from the Global Alzheimer's Platform (GAP) foundation, with known amyloid PET status. We evaluated SPEAR pTau 217 for its ability to differentiate between PET negative and positive donors, comparing sensitivity, specificity, and fold-change to the Eli Lilly MSD assay **Result:** SPEAR pTau 217 quantified all samples above the functional lower limit of quantitation (LLoQ), whereas the Eli Lilly MSD assay quantified 88% of samples (2% above the upper limit of quantitation (ULoQ); 10% below the LLoQ). SPEAR demonstrated nearly double the fold increase of PET positive results over PET negative compared to the Eli Lilly assay, with a 4.7-fold increase versus 2.5-fold. A single cutoff yielded 89.5% sensitivity, 95.5% specificity, 96.2% positive predictive value (PPV), and 87.5% negative predictive value (NPV). Optimizing for specificity and sensitivity to be 95% or higher for both Eli Lilly and SPEAR pTau 217 exhibited a reduction from 24.8% to 15.7% for the indeterminant range with the SPEAR assay. **Conclusion:** SPEAR UltraDetect™ pTau 217 exhibited exceptional analytical sensitivity, quantifying all tested samples and providing clear clinical differentiation between PET positive and negative cases with a 4.7-fold change. Clinical sensitivity and specificity was greater than 90% with negligible indeterminant range of 2% and when optimized for 95% resulted in a 1.6-fold reduction of indeterminant range as compared with Eli Lilly MSD. Its features, including 25-100 times less sample volume, a wash-free workflow, and compatibility with standard qPCR instruments, make SPEAR a robust, accessible, and scalable tool for neurological research and clinical diagnostics.

Disclosures: C. Worsfold: A. Employment/Salary (full or part-time): Spear Bio. **C. Fan:** A. Employment/Salary (full or part-time): Spear Bio.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.20/Web Only

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

Support: GR1069104 IS - ADRC Consortium for Clarity in ADRD Research Through Imaging (CLARiTI) - 23-8660-A0002-0
U24AG074855 - ADSP-PHC
U01-AG082350 - CLARiTI

Title: Linking brain atrophy subtypes to neuropathology in mild cognitive impairment and alzheimer's disease

Authors: *C. OWENS-WALTON[1], T. J. HOHMAN[4], S. C. JOHNSON[5], E. C. MORMINO[6], D. TOSUN[7], L. AKSMAN[2], P. M. THOMPSON[3];
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Res. Ctr., Univ. of Wisconsin, Madison, WI; [6]Dept. of Neurol. and Neurolog. Sci., Stanford, Stanford, CA; [7]Dept. of Radiology and Biomed. Imaging, Univ. of California San Francisco, San Francisco, CA

Abstract: Dementia places a significant burden on patients and caregivers. High levels of disease heterogeneity contribute to this burden, complicating patient management and impeding clinical trial design. This heterogeneity may be linked to the co-occurrence of primary Alzheimer's disease (AD) neuropathology (Amyloid-β and Tau) with other neuropathologies. Here we used Subtyping and Stage Inference (SuStaIn), an unsupervised machine-learning technique, to investigate brain-atrophy subtypes in individuals with clinical diagnosis of mild cognitive impairment (MCI) and dementia due to AD, and how subtypes differ on autopsy-derived neuropathology measures (binary presence of α-synuclein, hippocampal sclerosis, TAR DNA-binding protein 43 (TDP-43) and cerebral amyloid angiopathy). The SuStaIn algorithm incorporated FreeSurfer-derived brain MRI features from participants in the ADSP Phenotype Harmonization Consortium (training set: n=1,994; age: 75.2 ± 8.9 years; 51% female; MCI/AD 1,209/785). A subset of participants with available post mortem neuropathological data was analyzed as a held-out Neuropath test set (n=172; age: 80.0 ± 12.6; 46% female; MCI/AD 114/58). Three brain atrophy subtypes were found in the training set (Fig 1); these model parameters were then used to predict the subtype and stage of those in the Neuropath test set. Subtypes include 'typical' (S1), 'cortical' (S2) and 'subcortical' (S3), with subtype labels indicating where atrophy begins in the brain. Chi-squared tests found S1 had a significantly greater proportion of hippocampal sclerosis cases relative to S2, while S1 and S2 had significantly greater proportion of TDP-43 cases relative to S3. Our results suggest an important role for hippocampal sclerosis in the typical AD subtype, relative to the cortical subtype. Our results also further tie TDP-43 to greater brain atrophy in limbic cortical regions in MCI and AD. Future studies will investigate the link between subtypes and neuropathology data that encapsulates neuroanatomical location at a finer-grained level.

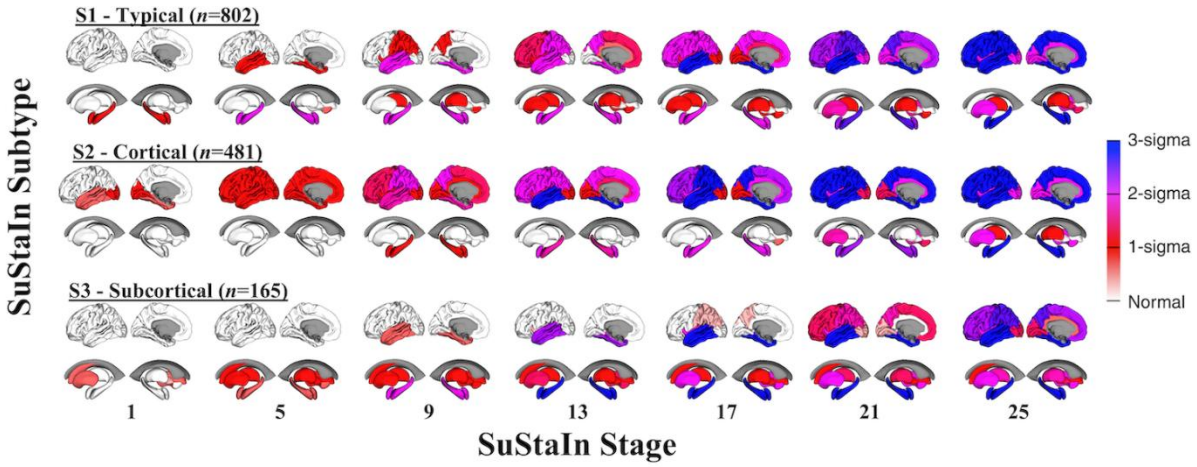

Fig 1. Three subtype progression patterns are shown on a model brain, with color representing cumulative (left-to-right) atrophy events in red (mild atrophy, z=1), magenta (moderate atrophy, z=2) and blue (high atrophy, z=3). SuStain FreeSurfer input features were adjusted for age, sex and intracranial volumes, then averaged bilaterally across regions: accumbens, amygdala, caudate, hippocampus, pallidum, putamen, thalamus, temporal lobe, frontal lobe, parietal lobe, cingulate, occipital lobe and insula cortex.

Disclosures: C. Owens-Walton: None. **T.J. Hohman:** Other; Scientific Advisory Board for Vivid Genomics, Consultant for Circular Genomics, Deputy Editor of Alzheimer's &

Dementia. **S.C. Johnson:** None. **E.C. Mormino:** None. **D. Tosun:** None. **L. Aksman:** None. **P.M. Thompson:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.21/F9

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

Title: Reciprocal Interactions Between Periodontal Disease and Alzheimer's Disease: Implications for Mutual Triggering, Exacerbation, and Treatment Interventions

Authors: *S. AL-GHARAIBEH, A. GHARAIBEH;
Insight Res. Inst., Flint, MI

Abstract: Periodontal health is connected to many systemic diseases, such as cardiovascular, diabetes mellitus, and neurodegenerative diseases. The oral-brain axis has gained increasing interest in the pathogenesis of diseases. Emerging studies have highlighted the potential role of periodontal disease in the development and progression of Alzheimer's disease. However, Alzheimer's disease also affects periodontal disease and oral health. In this study, we address the correlation between the two diseases and the mechanisms by which one contributes to the other. Exploring the correlation between Alzheimer's disease and periodontal disease will assist in better understanding the pathophysiology of diseases and pave the way for the development of therapeutic and preventive strategies

Disclosures: S. Al-Gharaibeh: None. **A. Gharaibeh:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.22/F10

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

Support: NIH Grant P30AG066530
NIH Grant P01AG052350
NIH Grant R01NS034467

Title: A specific and sensitive assay for the detection of APOE4 carrier status and protein levels in biofluids

Authors: *A. P. SAGARE[1,2], E. B. JOE[3], H. C. CHUI[3], J. M. RINGMAN[3], M. G. HARRINGTON[3], L. S. SCHNEIDER[3], H. N. YASSINE[3], C. TORRES SEPULVEDA[1,2], G. SPILLARD[4,2], B. V. ZLOKOVIC[7,2], D. A. NATION[5], A. W. TOGA[6], R. A. RISSMAN[8]; [2]Dept. of Physiol. and Neurosci., [3]Dept. of Neurol., [4]Physiol. and Neurosci., [5]Leonard Davis Sch. of Gerontology, [6]USC Stevens Neuroimaging and Informatics Inst., [1]USC, Los Angeles, CA; [7]Zilkha Neurogenetic Inst., Keck Sch. of Med. of USC, Los Angeles, CA; [8]Physiol. and Neurosci., Alzheimer's Therapeut. Res. Institute, Univ. of Southern California, San Diego, CA

Abstract: The apolipoprotein E4 (*APOE4*) allele is the strongest genetic risk factor for Alzheimer's disease (AD), increasing risk by about 4-fold with one copy and 12-fold with two copies compared to *APOE3* homozygous carriers. The *APOE4* allele also increases the risk of cerebrovascular dysfunction, including the risk for cerebral amyloid angiopathy (CAA) and a higher rate of amyloid-related imaging abnormalities (ARIA) from anti-amyloid antibody therapy. Studies suggest that higher *APOE4* expression contributes to the higher risk of AD. We developed a rapid, specific, one-step quantitative Meso Scale Discovery (MSD) sandwich ELISA assay to detect *APOE4* carrier status and analyze APOE4 levels in human plasma and cerebrospinal fluid (CSF) samples. We validated the assay by determining detection limits, dilution linearity, and precision. The purified recombinant APOE4 standard protein exhibited a linear curve fit ranging from 0.625 to 50 ng/ml, whereas recombinant APOE2 and APOE3 proteins were undetectable with this assay. CSF samples diluted from 1:40 to 1:100 and plasma samples diluted from 1:1000 to 1:2000 from *APOE4/4* and *APOE3/E4* carriers showed linear response curves across the dilution range, indicating the absence of matrix effects. The intra-assay coefficient of variation for both the samples and APOE4 standards was less than 5%. In summary, the optimized APOE4-specific MSD assay may be helpful for (1) rapid screening of patients in the clinic for APOE4 carrier status using one microliter of plasma or CSF in less than 2 hours, (2) measuring levels of APOE4 in biofluids to understand AD risk in different ethnicities, (3) analyzing steady-state levels of APOE4 in the CSF or plasma after therapeutic intervention aimed at reducing *APOE4* expression.

Disclosures: A.P. Sagare: None. **E.B. Joe:** None. **H.C. Chui:** None. **J.M. Ringman:** None. **M.G. Harrington:** None. **L.S. Schneider:** None. **H.N. Yassine:** None. **C. Torres Sepulveda:** None. **G. Spillard:** None. **B.V. Zlokovic:** None. **D.A. Nation:** None. **A.W. Toga:** None. **R.A. Rissman:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.23/F11

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

Support: Korea Institute of Oriental Medicine, funded by the Korean Government (KSN2312022)
Ministry of Health & Welfare in Republic of Korea (HF23C0010)

Korea Brain Research Institute, funded by the Ministry of Science and ICT (24-BR-03-05)

Title: Trial-to-trial sensory auditory event-related potential variability as a marker of cognitive impairment

Authors: *J. EYAMU[1,2];
[1]Digital health research division, Korea Inst. of Oriental Med., Daejeon, Korea, Republic of;
[2]KM Convergence Sci., Univ. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: *Background:* Mild cognitive impairment (MCI) is a preceding state of dementia and poses a significant health and economic burden globally. Timely detection of MCI offers the potential to slow disease progression and mitigate the emotional and financial burdens on patients and caregivers. Since early sensory input processing is automatic, sensory event-related potentials (ERPs) can be suitable for experiments involving the elderly, who often have diminished attention spans. The primary aim of this study was to examine sensory-perception deficits and fluctuations in MCI using auditory ERP obtained from a prefrontal two-channel EEG device.*Methods:* This study examined ERP and trial-to-trial variability in sensory neural responses to 8 various auditory sounds in 573 patients with MCI and 1,295 age-matched cognitively normal (CN) individuals. The trial-to-trial variability measures were calculated using the response variance curve (RVC). Cognitive decline was assessed using the Seoul Neuropsychological Screening Battery (SNSB) and the Mini-Mental State Examination (MMSE). Statistical analyses, such as independent t-tests, and univariate and multiple logistic regression were performed.*Results:* Independent of stimulating auditory frequencies, MCI patients showed significant differences in trial-to-trial variabilities in ERP amplitudes and area under the curves (AUCs) in sensation (N1) and perception (P2) processing while no such difference existed in the corresponding ERP amplitudes and AUCs. This was profound in the MCI participants in their 70s. Furthermore, unlike in sensory ERP measures, the trial-to-trial variability in MCI remained significant even after controlling for potential influences such as age, sex, years of education, and MMSE scores.*Discussion:* The instability in sensation-perception processing seen in patients with MCI, compared to CN, may indicate deficits in pre-attentive auditory stimuli detection and higher-order perceptual processing of external stimuli. The significant difference in MCI patients in and only in their 70s implies that this age group is in a transit state associating these intra-variabilities in sensation and perception with actual cognitive impairment. Considering the substantial sample size in this study and the reliance of current neuropsychological tests on cognitive and attention capabilities, these results underscore the importance of incorporating sensory ERP measures for MCI screening to supplement traditional neuropsychological tests.

Disclosures: J. Eyamu: None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.24/F12

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

Support: NIH Grant 1RF1AG079303-01A1

Title: Influence of Traumatic Brain Injury and APOE-ε4 on Alzheimer's-Related Proteins in Circulating Neuron-Derived Extracellular Vesicle Cargo

Authors: *M. CULLUM-DOYLE[1,2], M. S. PANIZZON[3], V. B. RISBROUGH[3], R. RISSMAN[2];
[1]Neurosciences, UCSD, La Jolla, CA; [2]Physiol. and Neurosci., Alzheimer's Therapeut. Res. Institute, Univ. of Southern California, San Diego, CA; [3]Psychiatry, Univ. of California San Diego, La Jolla, CA

Abstract: Background: Alzheimer's disease (AD) and traumatic brain injury (TBI) often co-occur, with studies showing higher AD prevalence among those with TBI history. This project investigates the relationship by examining biomarkers in the cargo of extracellular vesicles. While CSF and plasma biomarkers detect acute TBI, markers that persist long after the injury remain elusive. Neuron-derived extracellular vesicles (NEVs) from plasma carry AD-related proteins, such as amyloid-beta (Aβ), potentially offering greater sensitivity than free plasma proteins to chronic neuronal damage. Using biobanked plasma from the Vietnam Era Twin Study of Aging (VETSA3), we hypothesized that individuals with a history of TBI exhibit elevated AD-related proteins in NEV cargo. **Methods:** Plasma from 1,067 male twins aged 61-73 (mean age, 68) in VETSA3, approximately 40% of whom had mild to moderate TBI, was analyzed. NEVs were isolated via ExoQuick and magnetic immunocapture targeting L1CAM (CD-171). NEVs were characterized according to International Society for Extracellular Vesicles standards using nanoparticle tracking (NanoSight), ONI Nanoimaging, and a Flotillin-1 ELISA. AD-related proteins—Aβ40, Aβ42, total tau, pTau-181, and neurofilament light chain (NfL)—were quantified by SIMOA assays, normalized to flotillin, and log-transformed. Statistical models adjusted for age, testing site, race/ethnicity, and creatinine. Associations between NEV biomarkers, APOE genotype, and TBI characteristics were compared with those of non-TBI participants. **Results:** APOE-ε4 carriers with a history of TBI had significantly elevated NfL, total tau, Aβ40, and Aβ42 levels, indicating greater axonal injury and amyloid accumulation. TBI-negative APOE-ε4 carriers showed reduced pTau-181 versus non-carriers, suggesting altered tau phosphorylation. Aβ40 and Aβ42 were elevated in APOE-ε4 carriers, regardless of TBI, but were highest in those with a history of TBI. These results suggest a synergistic effect of APOE-ε4 and TBI on neurodegeneration, potentially accelerating Alzheimer's pathology. **Conclusions:** APOE-ε4 carriers with a history of TBI exhibit elevated AD-related proteins in NEVs, supporting a synergistic effect on neurodegeneration. NEV cargo proteins show promise as sensitive biomarkers for chronic neuronal damage influenced by genetic risk and brain injury.

Disclosures: M. Cullum-Doyle: None. **M.S. Panizzon:** None. **V.B. Risbrough:** None. **R. Rissman:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.25/F13

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

Support: NIH RF1 AG077658
NIH RF1 AG082030

Title: Early Neuronal Injury Identified with quantitative Gradient Recalled Echo (qGRE) MRI Coincides with Preclinical Amyloid Accumulation

Authors: *S. KOTHAPALLI[1], T. BENZINGER[1,3,4], R. J. BATEMAN[2,3,4], M. LI[2], C. ELDENIZ[1], J. C. MORRIS[2,3,4], D. A. YABLONSKIY[1,3,4];
[1]Radiology, [2]Neurol., Washington Univ. Sch. of Med., Saint Louis, MO; [3]Knight Alzheimer Dis. Res. Center, Washington Univ. in St. Louis, Saint Louis, MO; [4]The Hope Ctr. for Neurolog. Disorders, Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Background: Early identification of neuropathological changes in Alzheimer's disease (AD) is essential for disease-modifying therapies. Either β-amyloid (Aβ) elevation in the brain or reduction in CSF are considering the earliest AD biomarkers, and both are indirectly associated with neurodegeneration. Brain MRI volumetric measures the direct neurodegeneration. Herein, we propose a quantitative Gradient-Recalled-Echo (qGRE) MRI method, providing a sensitive biomarker (Neuronal Density Index, NDI) for quantifying the neurodegeneration before it can be detected by volumetric measurements. **Methods**: 143 participants, ages 48-88y, recruited through the Knight ADRC and SEABIRD study, provided informed consent. qGRE and PET amyloid ^{11}C-PIB images were acquired concurrently. For each participant, amyloidosis level was identified by mean-cortical-standard-uptake-ratio (mcSUVr), while NDI was quantitated in all cortical FreeSurfer-defined brain regions. Group differences between participants' NDIs were established for a range of mcSUVR cutoff levels. **Results**: Results in Figure 1 show dramatic pre-atrophic neurodegeneration in Aβ(+) group with NDI significantly decreased in multiple brain regions, while significant volume changes were observed only in frontal and insular regions. Furthermore, the NDI exhibited significant group differences in multiple brain regions even when mcSUVR cut-off threshold was reduced to 1.2. Importantly, NDI exhibited significant changes in medial temporal lobe even when comparing groups with mcSUVr < 1.2 and mcSUVR between 1.2 and 1.6. Volume measurements did not show significant differences until participants with mcSUVR greater than 3.4 were included in the analysis. **Conclusion**: qGRE-based NDI provides in-vivo microstructural brain neurodegeneration distribution in early amyloid pathology where atrophy is not detectable. While NDI reflects neuronal density, inflammation may also lower its value. Further study is needed to clarify the microstructural basis of pre-atrophic neurodegeneration.

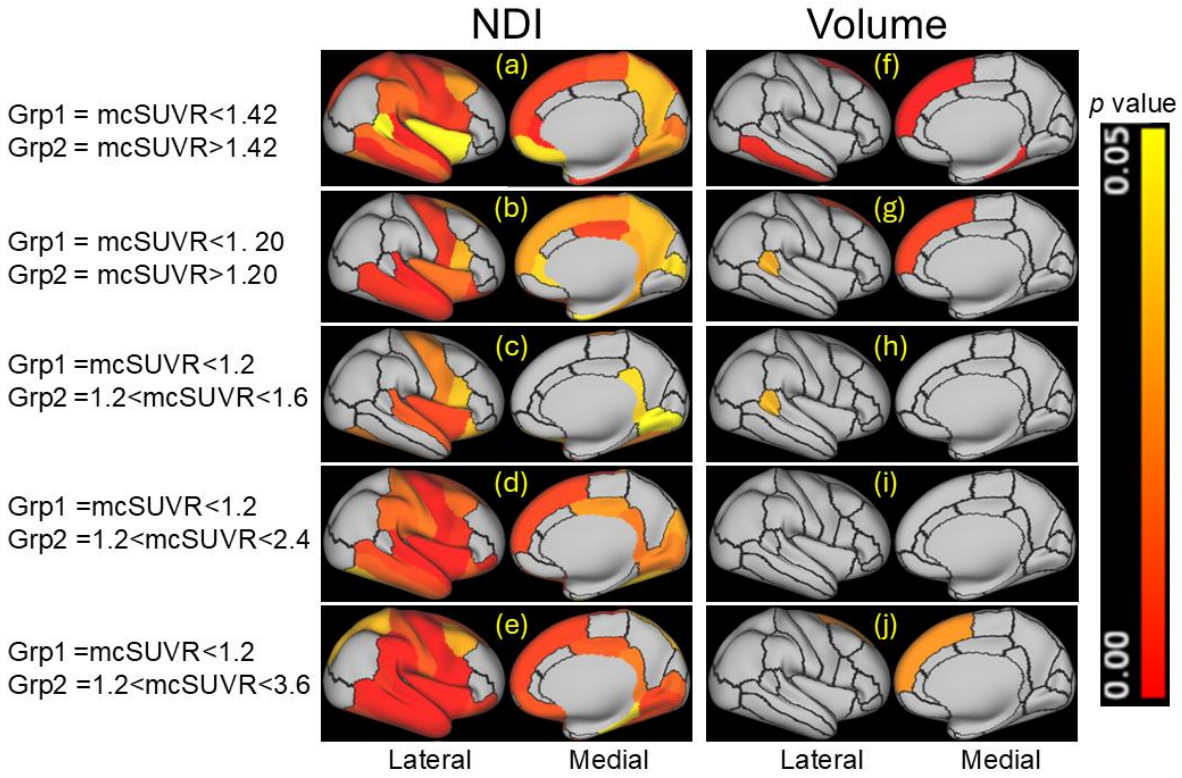

Figure 1. Surface maps illustrating the significance level of group differences in NDI (left column) and volumes (right column) across multiple cortical regions. Rows correspond to the groups separated at different PiB mcSUVR cutoffs. Grp1 – group of participants with mcSUVR less than cutoff, Grp2 – group of participants with mcSUVR exceeding the cutoff (rows 1 & 2) and group of participants with mcSUVR between 1.2-1.6 (row 3), between 1.2-2.4 (row 4), and between 1.2-3.6 (row 5).

Disclosures: S. Kothapalli: A. Employment/Salary (full or part-time): Washington University in St. Louis. **T. Benzinger:** A. Employment/Salary (full or part-time): Washington University in St. Louis. 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 or contracts from any entity, Siemens, payments to institution. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Other research support includes Hyperfine, scanner loan to institution; Lantheus, LMI, and Avid Radiopharmaceuticals/Eli Lilly, technology transfer and precursors for radiopharmaceuticals. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Fees include travel reimbursements from Cedars Sinai Medical Center, Hong Kong Neurological Association, and Alzheimer's Association. F. Consulting Fees (e.g., advisory boards); Fees include consulting fees

from Biogen, Eli Lilly, Eisai, Bristol Myers Squibb, J&J and Merck. **R.J. Bateman:** A. Employment/Salary (full or part-time): Washington University in St. Louis. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); equity ownership interest in C2N Diagnostics and income based on technologies licensed by Washington University to C2N Diagnostics. **M. Li:** A. Employment/Salary (full or part-time): Washington University in St. Louis. **C. Eldeniz:** A. Employment/Salary (full or part-time): Washington University in St. Louis. **J.C. Morris:** A. Employment/Salary (full or part-time): Washington University in St. Louis. F. Consulting Fees (e.g., advisory boards); BarcelonaBeta Brain Research Foundation Scientific Advisory Board, Native Alzheimer Disease-Related Resource Center in Minority Aging Research, External Advisory Board, Geriatric Emergency Care Applied Research (GEAR), Oversight Committee. **D.A. Yablonskiy:** A. Employment/Salary (full or part-time): Washington University in St. Louis.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.26/F14

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

Title: Biomarkers in Mild Cognitive Impairment using Resting-State EEG: Spectral, Aperiodic, Connectivity, and Microstate Analyses

Authors: *S. NAAZ[1], G. KUMAR[1], R. VENUGOPAL[1], A. SASIDHARAN[1], T. N. SATHYAPRABHA[1], S. PALANIMUTHU THANGARAJU[2], J. P. JOHN[2], B. M. KUTTY[1], P. N. RAVINDRA[1];
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Abstract: Introduction: Mild Cognitive Impairment (MCI) represents a transitional stage between normal aging and dementia, characterized by cognitive decline. EEG markers may provide insights into underlying neural mechanisms. This study aims to explore resting-state EEG in individuals with MCI compared to healthy age-matched controls. Specifically, we investigate resting-state EEG biomarkers in MCI, focusing on spectral power, aperiodic measures, connectivity, and microstate analysis. **Methods:** The study included 10 healthy controls and 9 individuals with MCI, aged 50 to 75 years. Resting EEG data were acquired using 19 electrodes (Nihon Kohden) for 6 minutes. Preprocessing was conducted using EEGLAB, and Welch's power spectral density (PSD) analysis was performed using MATLAB. Additionally, we analyzed aperiodic components using FOOOF and IRASA, along with connectivity and microstate analysis. Statistical analysis included descriptive statistics and t-tests conducted using Jamovi (v2.3.21), with a significance level set at $p = 0.05$. **Results**: PSD analysis did not show any differences between the MCI group and age-matched healthy controls. However, other measures revealed significant differences. Aperiodic analysis using FOOOF and IRASA showed

that delta power and the exponent at the central region, beta1 power at the temporal region, and beta2 power and spectral edge at the parietal region were reduced in MCI, whereas the offset at the temporal region was increased. Connectivity analysis indicated increased theta connectivity in the frontal, parietal, and temporal regions. Microstate analysis revealed that Global Field Power (GFP) in Microstates A, C, E, and G was increased in MCI, whereas Global Explained Variance (GEV) in Microstate D and Mean Spatial Correlation (MspatCorr) in Microstate D were reduced compared to healthy controls. **Discussion:** These findings suggest that region-specific alterations in aperiodic components, functional connectivity, and microstates, particularly in key areas involved in memory, executive function, and sensory integration, may indicate early compensatory mechanisms such as hyper-synchronization and hyperactivation.

Disclosures: S. Naaz: None. **G. Kumar:** None. **R. Venugopal:** None. **A. Sasidharan:** None. **T.N. Sathyaprabha:** None. **S. Palanimuthu Thangaraju:** None. **J.P. John:** None. **B.M. Kutty:** None. **P.N. Ravindra:** None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.27/F15

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

Support: SUPPORTED BY THE 2024-2.1.1-EKÖP-2024-00004 UNIVERSITY RESEARCH SCHOLARSHIP PROGRAMME OF THE MINISTRY FOR CULTURE AND INNOVATION FROM THE SOURCE OF THE NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND

Title: 1/f slope and alpha peak frequency as early eeg markers of hyperexcitability in mild cognitive impairment

Authors: *O. LANYI;
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Abstract: The aperiodic component of the EEG signal (1/f slope) has recently gained attention as a sensitive marker of cortical excitability, which has been associated with dementia and Alzheimer’s Disease. As dementia continues to pose a growing public health challenge, identifying early, non-invasive markers of neural dysfunction is critical for early diagnosis and intervention. In this study, we investigate whether the aperiodic exponent and individual alpha peak frequency are reliable markers to differentiate between healthy aging and Mild Cognitive Impairment (MCI). 31 MCI patients (17 non-amnestic MCI, aged 58-82, and 14 amnestic MCI, aged 55-79) and 16 healthy elderly individuals (HC, aged 51-83) were recruited in the Department of Psychiatry, Semmelweis University in Budapest, Hungary. All participants completed a comprehensive neuropsychological assessment, resting-state EEG (64-channel

Neuroscan) under both eyes-open and eyes-closed conditions (4 minutes each), and MRI scans. In addition, participants completed the Paired Associates Learning task (PAL) during EEG recording. EEG data were preprocessed using EEGLAB, and the SpecParam Python toolbox was used to fit the aperiodic slope. Preliminary results indicate lower individual alpha peak frequency and overall alpha power in temporal and parietal EEG channels in MCI compared to HC. The aperiodic exponent in the 1-45 Hz range showed a trend-level group difference. Ongoing analyses will assess whether a broader frequency range (0.5-100 Hz) reveals stronger effects. We are also investigating relationships between 1/f slope and working memory performance. While our preliminary results replicate the slowing of the alpha peak frequency in MCI the reliability of the aperiodic slope remains under investigation. The flattening of the 1/f slope could be suggestive of increased neural noise and cortical hyperexcitability in MCI; however, the 1-45 Hz frequency range is not sufficient to capture it.

Disclosures: O. Lanyi: None.

Poster

PSTR383: Alzheimer's Disease: Biomarkers

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR383.28/F16

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

Support: Spot Dx

Title: Alzheimer's Disease Detection via Zipf's Law

Authors: *F. V. CHIRILA;
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Abstract: Zipf's law,[1] a captivating phenomenon that states the frequency of an event is inversely proportional to its rank, was initially observed in the frequencies of words and the spatiotemporal network dynamics of city populations, reflecting the intricate patterns of human choices. Likewise, the spatiotemporal network dynamics of skin fibroblast aggregates envisioned as "cell cities" exhibit Zipf's law under specific conditions [3, 4]. Zipf's law has exciting applications in diagnostics, ranging from early indicators of cognitive impairment due to reduced lexical richness [5, 6] to cortical EEG recordings associated with neurological disorders, such as epilepsy or cognitive decline. [7, 8] Here we undertake a fascinating examination of how Zipf's law can revolutionize the diagnosis of Alzheimer's Disease (AD). Our findings reveal that skin fibroblasts from patients with AD, non-AD dementia, and healthy individuals display distinctive Zipf law parameters, paving the way for an accurate diagnosis. We established a diagnostic cutoff using a training set of 34 healthy controls, which we then applied to a robust validation set comprising 35 patients with Alzheimer's disease and 27 individuals with non-AD dementia. The Zipf biomarker results are striking; The overlap probability between Alzheimer's disease and non-Alzheimer's is less than 1.2×10^{-13} in a two-tailed unequal variance t-test; The sensitivity is

100.00% (95% C.I. 90.00-100.00%), and the specificity is 100.00% (95% C.I. 87.23% - 100.00%). Such outcomes underscore the remarkable promise of leveraging Zipf's law for the detection of Alzheimer's Disease. Zipf's biomarker outshines the Morphometric Imaging biomarker [3, 4], showcasing a 1.44-fold enhancement in the differentiation between averages for AD and non-AD dementia cohorts, alongside a remarkable 3.60-fold improvement in the gap distinguishing between these groups. The significant reduction in the risk of misdiagnosis, one in ten trillion ($P<1.2 \times 10^{-13}$}, is further illustrated by the heightened sensitivity and specificity observed in high-risk scenarios, all of which are validated by a stringent 95% confidence interval and confirmed by the gold standard autopsy in most samples. The Zipf biomarker's precision increased when compared with the Morphometric Imaging biomarker by an average of 9-fold in 34 healthy controls, with a range of 2 to 14, 9-fold in 35 Alzheimer's Disease patients, with a range of 3 to 26, and 10-fold in 27 non-Alzheimer's Disease dementia patients, with a range of 3 to 27. This study heralds a transformative approach to Alzheimer's diagnosis, blending statistical elegance with clinical precision.

Disclosures: F.V. Chirila: A. Employment/Salary (full or part-time): Spot Dx.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.01/G1

Topic: C.03. Parkinson's Disease

Support: Secretariat of Science Humanities Technology and Innovation, Basic and Frontier Science program (CBF2023-2024-1888)

Title: Neuroprotective effect of D pinitol in a murine model of Parkinsonism

Authors: *A. ALONSO-CASTRO[1], M. JUÁREZ VÁZQUEZ[2], M. GONZALEZ RIVERA[2], R. RAMÍREZ[2], A. MELECIO HERNÁNDEZ[2];
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Abstract: Parkinson's disease is the second most common neurodegenerative disorder worldwide, in which brain dopamine levels decrease by 80% to 90%. Our research group found that D-pinitol, a cyclic polyol, has anxiolytic, antidepressant, and anticonvulsant effects in mouse models. The neuroprotective effect of this compound has not been described and was evaluated in a mouse model with Parkinson's-associated symptoms caused by the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The Research Ethics Committee of the University of Guanajuato reviewed and approved the protocol for this study (CIBIUG-P40-2021). Groups (n=8) of male C57BL/6 mice from the vivarium of the Neurobiology Institute (UNAM) consisted of the following: a) Control: received oral saline for 28 days (total duration of the experiment). b) Vehicle: received five intraperitoneal (i.p.) administrations of MPTP (25 mg/kg) every 24 hours for five days. Then, the animals received oral saline for 23 days. c) Experimental

groups received five administrations of MPTP (i.p.) every 24 hours for five days. These animals received D-pinitol at 10, 50, and 100 mg/kg p.o. or 25 mg/kg p.o. L-Dopa for 28 days, including administration with MPTP. Each behavioral test (rotarod, tail suspension test, cylinder exploration test, and others) was performed one hour later after drug administration on days 17, 21, 24, and 28 of the experiment. The findings showed that the vehicle group (MPTP + saline) impaired ($p < 0.05$) the motor coordination of mice in the rotarod test at 0 h, 1 h, and 2 h, compared to the control group. The D-pinitol + MPTP treatment groups maintained motor coordination at doses of 10, 50, and 100 mg/kg (p.o.), in a similar way to the control group, whereas the L-dopa + MPTP group decreased motor coordination ($p < 0.05$) at 1 and 2 h post-treatment. In the tail suspension test, the vehicle group increased ($p<0.05$) the immobility time compared to the control group; only the 100 mg/kg D-pinitol + MPTP group decreased ($p<0.05$) the immobility time compared to the vehicle group in a similar way to the control group. In the exploratory cylinder test, the vehicle group increased the number of rearings compared to the control group, whereas the 50 and 100 mg/kg D-pinitol+MPTP reduced ($p<0.05$) the number of rearings. Current work is performed in our laboratory to understand the molecular mechanisms by which D-pinitol induces its neuroprotective effects. In conclusion, D-pinitol (50-100 mg/kg p.o.) protected mice from motor coordination imbalance, anxiety, and depression-like behavior promoted by the neurotoxin MPTP.

Disclosures: A. Alonso-Castro: None. **M. Juárez Vázquez:** None. **M. Gonzalez Rivera:** None. **R. Ramírez:** None. **A. Melecio Hernández:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.02/G2

Topic: C.03. Parkinson’s Disease

Title: Integrative systems biology and Bayesian artificial intelligence reveal therapeutic targets in Parkinson’s disease from the Living Brain Project

Authors: G. M. MILLER[1], N. MAHAVEER CHAND[1], C. BRUCE[1], S. GESTA[1], N. R. NARAIN[1], E. VORNHOLT[2], B. KOPELL[2], N. D. BECKMANN[2], A. CHARNEY[2], *M. A. KIEBISH[1];
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Abstract: Molecular characterization and therapeutic target discovery in neurodegenerative diseases often rely on postmortem tissue samples, which may not recapitulate the in vivo molecular phenotype due to confounding factors such as time of collection, tissue preservation, and postmortem molecular changes. Alternatively, prefrontal cortex tissue samples collected during routine deep brain stimulation (DBS) procedures from implantation of devices offer a unique opportunity for molecular discovery in living human brain tissue from snap frozen samples. Over the past decade, a repository of tissue samples with detailed clinical phenotyping,

electrophysiological data, neuroelectrochemical profiles, and cognitive assessment has been generated to form the basis of the Living Brain Project (LBP). Diagnoses among the LBP cohort included Parkinson's Disease (PD), Alzheimer's Disease, obsessive-compulsive disorder, and other neurological conditions. These samples were subjected to integrative molecular profiling, including genomics, transcriptomics, and proteomics. To identify novel therapeutic targets, we employed a Bayesian artificial intelligence system (NAi platform) to integrate transcriptomics and clinical data. A causal network map was generated from living subjects with PD (n = 220), and without PD (n = 55) from the LBP. A total of 21,647 data features including transcriptomics and clinical features were utilized for generation of the model network. To derive actionable insights from this complex network and identify potential therapeutic targets, we applied topological analysis, which uses the principles of graph theory to highlight network pressure points or hubs whose expression may exert system-side influence throughout the network. As an initial positive control validation, Glucocerebrosidase (GBA), a known risk factor for PD was investigated in our network and found to be connected to established biology associated with lysosomal and mitochondrial genes along with novel biology. Similarly, examination of the network neighborhood around known PD driver genes PRKN, SCNA, and PINK1 showed expected biology as revealed by pathway enrichment analysis. While GBA was found to be in the top 1% from the centrality analysis, our results highlighted additional highly ranked targets involved in protein aggregation and neurodegenerative diseases. Notably, one well-established PD target emerged as one of the most central nodes in the entire network, further supporting the model's validity. These candidate targets are currently undergoing bioanalytical and molecular validation to assess their therapeutic potential for the treatment of PD.

Disclosures: G.M. Miller: A. Employment/Salary (full or part-time): BPGbio. **N. Mahaveer Chand:** A. Employment/Salary (full or part-time): BPGbio. **C. Bruce:** A. Employment/Salary (full or part-time): BPGbio. **S. Gesta:** A. Employment/Salary (full or part-time): BPGbio. **N.R. Narain:** A. Employment/Salary (full or part-time): BPGbio. **E. Vornholt:** None. **B. Kopell:** A. Employment/Salary (full or part-time): BPGbio. **N.D. Beckmann:** None. **A. Charney:** None. **M.A. Kiebish:** A. Employment/Salary (full or part-time): BPGbio.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.03/G3

Topic: C.03. Parkinson's Disease

Support: Parkinson's Foundation Center Grant PF-RC-936279
NIH F31 Ruth L. Kirschstein National Research Service Award F31NS137751

Title: Profiling and characterizing a novel vulnerable neuron type in the substantial nigra of Parkinson's disease

Authors: *M. LIANG[1], E. LI[2], C. XIAO[1], M. WANG[3], Z. YUE[1];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Dalton High Sch., New York, NY; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY.

Abstract: Parkinson's disease (PD) is the second leading neurodegenerative disorder characterized by degeneration of neuromelanin-containing dopaminergic (DA) neurons in the substantia nigra (SN). Whether non-DA neurons are vulnerable in PD is poorly understood. We previously performed single-cell transcriptomics analysis of human SN including 9 healthy controls and 23 idiopathic PD. A combination of immunostaining and validation against datasets from independent cohorts resulted in the identification of molecularly distinct subtypes of DA-related neurons, including a RIT2-enriched population in aged human SN. RIT2 variants have previously been linked to PD. Validation in mouse and human SN identifies a RIT2 population that partially overlaps with TH, a marker for DA neurons, and the subpopulation (RIT2+/TH-) was found to be vulnerable in PD. By validation against independent datasets and using transgenic mouse models, we investigate distinct RIT2 neuronal subpopulations including its distribution, morphology by 2-photon imaging, cell identity by snRNAseq, and vulnerability to α-synuclein. Our study highlights the need to study non-DA neurons in the SN in order to understand the neuropathogenesis in PD.

Disclosures: M. Liang: None. **E. Li:** None. **C. Xiao:** None. **M. Wang:** None. **Z. Yue:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

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Topic: C.03. Parkinson's Disease

Support: The work was supported by the National Science Centre, Poland, under research project no 2017/25/B/NZ7/02795, awarded to Jaroslaw Marusiak
Internal scientific grant from the Wroclaw University of Health and Sport Sciences, awarded to Jaroslaw Marusiak

Title: Aerobic interval training enhances neuroplasticity and motor function in Parkinson's disease

Authors: *J. MARUSIAK;
Wroclaw Univ. of Hlth. and Sport Sciences, Fac. of Physiotherapy, Dept. of Physiothera, Wroclaw, Poland

Abstract: ***Background:*** Animal models suggest that intensive physical training may promote neuroplasticity in Parkinson's disease (PD), but its effects in humans remain underexplored. ***Objectives:*** To investigate the effects of aerobic interval training (AIT) on brain-derived neurotrophic factor (BDNF) levels, striatal [^{18}F]fluorodopa PET/CT uptake ([^{18}F]DOPA$_{PET/CT}$), electroencephalography (EEG), and motor function in PD patients, and explore their

relationships to understand underlying mechanisms. ***Methods:*** Thirty PD patients were randomly assigned to: (1) the training group (PD-TR, n=15) with a 12-week moderate-intensity AIT program, and (2) the non-training group (PD-NTR, n=15). Assessments were conducted pre- and post-12-week period, including BDNF levels, striatal [^{18}F]DOPA$_{PET/CT}$, EEG during motor task-evoked desynchronization (EEG-ERD$_{MT}$) and rest-evoked synchronization (EEG-ERS$_{REST}$) in primary motor cortex (M1) and supplementary motor area (SMA). Motor function was evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS), as the total UPDRS Part III score and UPDRS based Manual Bradykinesia Score for the Affected Upper Extremity (MBS-AUE), which was calculated as the sum for the scores of assessing Finger Tapping (UPDRS 3.4), Hand Movements (UPDRS 3.5), and Pronation-Supination tasks (UPDRS 3.6). ***Results:*** The training effects in the PD-TR group included increased BDNF levels ($p < 0.001$) and improved motor function, including total UPDRS Part III score ($p < 0.05$) and MBS-AUE score ($p < 0.05$). Both groups showed increased EEG-ERS$_{REST}$ M1 ($p < 0.05$) over time, with no changes in striatal [^{18}F]DOPA$_{PET/CT}$ uptake. There were significant inter-group (PD-TR vs. PD-NTR) differences in the correlation values of: (i) ΔBDNF with Δ[18F] DOPA$_{PET/CT}$ uptake in the putamen ($p < 0.001$) and with ΔEEG-ERD$_{MT}$ M1 ($p < 0.001$); (ii) Δ[18F] DOPA$_{PET/CT}$ uptake in the putamen with Δ[18F] DOPA$_{PET/CT}$ uptake in the caudate ($p < 0.05$), as well as with ΔEEG-ERS$_{REST}$ SMA ($p < 0.05$); (iii) ΔMBS-AUE scores with ΔEEG-ERS$_{REST}$ SMA ($p < 0.001$). ***Conclusions:*** A 12-week AIT enhanced BDNF levels and motor function in PD-TR, with neuroplasticity driven central nervous system contributions supporting this motor recovery.

Disclosures: J. Marusiak: None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.05/G5

Topic: C.03. Parkinson's Disease

Support: MJFF- 4273310

Title: Mechanisms driving the cognition enhancing action of glycosylated pna5 for parkinson's disease

Authors: *K. BERNARD;
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Abstract: Parkinson's Disease (PD) is a chronic age-related neurodegenerative disease. While clinical diagnosis is based upon the presence of the hallmark motor symptoms, non-motor symptoms such as cognitive decline are a prominent feature of disease. Currently, there are no treatments that can intervene in disease progression, nor satisfactorily address PD associated cognitive decline. From this perspective, we designed and synthesized a glycosylated Ang(1-7) analog (PNA5) that shows increased **peptide stability and enhanced CNS penetration. We've**

previously demonstrated that PNA5 improves cognition in the Thy-1αSyn (hαSyn) mouse model after 2 months of treatment through reduction of microglia-mediated neuroinflammation (Bernard et al., Experimental Neurology, 2024). This was accompanied by a reduction in the peripheral levels of MIP-1β, a cytokine linked to neurodegeneration. Our current study aims to 1) study the transcriptional mechanisms underlying PNA5 action and (2) delineate the role of MIB-1β in the PNA5 neuroprotection and validate its use as a potential biomarker for target engagement, in both males and females. We are applying PNA5 at the previously used 1 mg/kg (*s.c.*) dose, as well as 0.5 mg/kg. Furthermore, we are collecting behavioral data prior to PNA5 treatment (~4 months of age), as well as after 2 months of PNA5 or saline treatment (~6months of age) to evaluate changes over time and to determine if PNA5 is able to reverse deficits already present or is mainly acting to prevent further decline. At start, we see a decrease in the spontaneous alteration of male hSyn animals relative to WT counterparts (63.7% vs. 53.6%, $p = 0.0082$), indicating a cognitive deficit present at baseline. Conversely, there is no difference between female animals, (56.5% vs. 61.1%, $p = 0.15$). Female mice display a mild, but non-significant reduction in shredding behavior at the 4-month baseline timepoint (16.34g vs. 12.34 g, $p = 0.057$), while males have a pronounced deficit at this time point (21.87 g vs. 2.452 g, $p < 0.0001$). Analysis of post-treatment behavior is underway and will include cognitive tasks such as the novel object recognition, spontaneous alteration in a y-maze, and motor specific behaviors such as the nestlet and challenging beam task. We are also assessing the animals via immunohistochemistry (n = 10/group), RNAseq analysis (n = 10/group), and plasma level analysis for molecular pathway and biomarker identification. These studies will elucidate the transcriptional and mechanistic changes associated with PNA5 mediated cognitive enhancement, which will help provide targets for further biomarker development.

Disclosures: K. Bernard: None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.06/G6

Topic: C.03. Parkinson’s Disease

Support: NS123405

Title: Dj-1 is required for local protein synthesis and growth of distal processes of midbrain dopaminergic neurons

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Abstract: Hallmarks of Parkinson’s Disease (PD) are mitochondrial dysfunction and proteostasis defects. Dopaminergic neurons have long neuritic arbors that release dopamine in the striatum. Although local protein synthesis is likely required for growth and maintenance of

this extensive network, this has not been shown previously. We studied patient mutant fibroblasts and isolated rodent DJ-1-/- mesencephalic dopaminergic neurons by using cellular techniques including imaging, RNA scope and puromycinPLA. We show that the familial PD risk gene PARK-7 (DJ-1), reported to affect mitochondrial function, regulates protein synthesis rates in human fibroblasts and rodent mesencephalic dopaminergic neurons. We find that DJ-1 acts as an mRNA binding protein (RBP) for F1FO ATP synthase β subunit mRNA (ATP5b). ATP β protein is the major component of the soluble ATP synthase F1 subcomplex, but its expression is low in DJ1-/- neurons and patient fibroblasts. In keeping with this, in DJ1 lacking cells, we find low overall protein synthesis rates and reduced growth, while in contrast overexpression of ATP β improves protein synthesis rates in patient fibroblasts and DJ- 1-/- neurons, increases the amount of ATP β in the distal neurites and rescues the growth of the neuritic processes. In conclusions, DJ-1 improves growth by affecting the amount of local ATP synthase assembly in the neuronal processes and this is required for ATP synthase dependent local protein synthesis. Our findings uncover a novel function for ATP β in local mRNA translation and demonstrate a crucial role for DJ-1 chaperone function in optimizing the stoichiometric structure of the ATP synthase to support neuronal growth.

Disclosures: R. Chen: None. **P. Licznerski:** None. **J. Chen:** None. **W.J. Mandemakers:** None. **E.A. Jonas:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

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Program #/Poster #: PSTR384.07/G7

Topic: C.03. Parkinson’s Disease

Support: ES024745-06
ES033462-0

Title: Characterizing an Interaction between Acyl-CoA: Cholesterol Acyltransferase 1 and the NLRP3 Inflammasome

Authors: *E. N. FIKSE[1,2], K. BIGGS[1,2], C. CHANG[2], M. C. HAVRDA[1,3];
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Abstract: The NLRP3 inflammasome detects cellular damage, such as endoplasmic reticulum (ER) stress and lysosomal dysfunction, initiating a neuroinflammatory response. It has emerged as a key area of interest in Parkinson's disease (PD) research; however, our understanding of its role in the brain remains limited. Activated inflammasomes cleave pro-inflammatory cytokines while increasing secretory activity, potentially leading to pyroptosis—a type of lytic and inflammatory cell death. Lipids play a role in endolysosomal homeostasis and neuroinflammation, but the molecular interactions between these cellular processes are not well

understood. Cholesterol metabolism and endolysosomal proteostasis are disrupted in Parkinson's disease. Parkinson-related proteinaceous stress activates inflammasomes, and NLRP3-mediated inflammation relies on intracellular cholesterol levels. Intracellular cholesterol is essential for maintaining the integrity of membrane-bound organelles, such as the ER and lysosomes. Acyl-CoA: Cholesterol Acyltransferase 1 (ACAT1), located in the ER, is responsible for cholesterol esterification and storage. Inhibiting ACAT1 increases cholesterol availability. Genetic models that elevate cholesterol in mouse brains have demonstrated a reduction in Alzheimer's-related pathology, including aggregated amyloid and tau; however, these models have not yet been explored in the context of NLRP3-related neuroinflammation and Parkinson's disease (PD). Our work predicts that microglia depend on free cholesterol to degrade disease-related protein aggregates and prevent neurotoxic pyroptotic death. Here, we employ genetic and pharmacological methods to inhibit ACAT1 and investigate whether increasing intracellular cholesterol suppresses NLRP3 activity. In purified microglial cultures exposed to canonical inflammasome activators, inhibiting ACAT1 resulted in decreased release of activated IL-1β and Gasdermin D, direct targets of the NLRP3 inflammasome. Interestingly, *Nlrp3* loss suppressed secretion, while *Acat1* loss enhanced secretion. Furthermore, when co-cultured with astrocytes, we again observed an exaggerated Acat1-dependent secretory phenotype. To further investigate the complexities of inflammasome-related secretion, we used RNAScope in situ hybridization and immunohistochemistry to demonstrate Acat1 expression in both microglia and astrocytes in vivo. Ongoing work will characterize the interactions between cholesterol homeostasis, inflammation, and proteinopathy, aiming to understand and modify microglial function in the context of PD and related disorders.

Disclosures: E.N. Fikse: None. **K. Biggs:** None. **C. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Application Entitled: Method for Attenuating Neuroinflammation, Amyloidopathy and Tauopathy. **M.C. Havrda:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR384.08/G8

Topic: C.03. Parkinson's Disease

Support: FONDECYT de Postdoctorado No. 3230347

Title: Neuroprotective effect and antioxidant activity of Parastrephia quadrangularis from Atacama Desert: Evidence from a Drosophila model of Parkinson's disease

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by the selective loss of dopaminergic neurons (DNs) from *Substantia Nigra* and the accumulation of toxic intracellular aggregates rich in α-synuclein. Patients exhibit both non-motor and motor symptoms (MS), the latter emerging following significant loss of DNs and dopamine depletion in *striatum*. Oxidative Stress (OxS) is a major contributor to DN vulnerability in PD. Current treatments alleviate MS instead of halting neuronal degeneration, underscoring the urgent need for alternative therapies able of preventing, delaying or halting neurodegeneration. Exogenous antioxidants have emerged as a strategy to neutralize radical species implicated in OxS. Plant-derived antioxidants, such as flavonoids, have been shown to scavenge reactive species and modulate key signaling pathways such as NF-kB, STAT and Nrf2. *Parastrephia quadrangularis* (*Pq*), an endemic plant from Atacama Desert with traditionally used for medicinal purposes, contains high levels of flavonoids and p-hydroxyacetophenone derivatives, suggesting its potential antioxidant and anti-inflammatory activities. However, its neuroprotective capacity remains unexplored. This study aims to assess the *in vitro* antioxidant capacity and *in vivo* neuroprotective effects of *Pq*. The hydroalcoholic extract (HAE) of *Pq* was obtained by maceration. Sub-fractions were obtained via chromatographic separation using an eluotrophic series of solvents. The total polyphenol and flavonoid content were measured, and the *in vitro* antioxidant activities were screened using FRAP, DPPH and ABTS assays. HAE-*Pq* exhibited the highest *in vitro* antioxidant capacity and showed no cytotoxic effects in SHSY-5Y cell culture. To assess neuroprotective effects *in vivo*, HAE-*Pq* was administered to a *Drosophila* PD model induced by α-synuclein overexpression in DNs. Control (*th*>+) and PD (*th*>*SNCA.J*) flies were exposed to HAE-*Pq* from larval stages to 7-9 days of adulthood. Climbing assays showed that HAE-*Pq* delayed the onset of motor deficit by one week in female PD flies (n=100, $p<0.05$). These findings suggest that *Pq* may exert a neuroprotective effect in a PD fly model, likely through its antioxidant activity. It also highlights the use of *Drosophila* as *in vivo* platform for rapid screening of potential therapeutic compounds for PD and related diseases.

Disclosures: R.V. Zárate: None. **B.I. Cárdenas:** None. **A.P. Cuevas:** None. **P. zamorano:** None. **A. Paredes:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.09/G9

Topic: C.03. Parkinson's Disease

Title: Natural Defense Against Neurodegeneration: Tetrahydrocurcumin Role in Neuroprotection

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Abstract: One of the most advantageous natural compounds utilized worldwide is *curcumin*, often known as *curcuma longa* or turmeric, because of its strong pharmacodynamic qualities with minimal side effects. *Curcumin* has been utilized both prophylactically and therapeutically as a nutraceutical and dietary supplement. Additionally, it has also been used for cosmetic purposes. The low bioavailability of Curcumin, on the other hand, has long minimized its use in healthcare. As a result, research on curcumin's metabolites has recently gained prominence, and several studies have been conducted to improve curcumin utilization. Curcumin or curcuminoid metabolites also exhibit biological activity that is equivalent to or superior to that of their precursor, according to past and current studies. The current study aimed to establish novel neuroprotective activities of Tetrahydrocurcumin (a key curcumin metabolite). To elucidate and validate the neuroprotective properties of Tetrahydrocurcumin along with potential neuroprotective mechanisms, both *in-silico* and *in-vitro* studies were carried out. The effect of tetrahydrocurcumin on the viability of hippocampus and dopaminergic neurons was demonstrated using HT-22 (hippocampal neurons) and N27 (dopaminergic neurons). Additionally, to study the neuroprotective mechanisms, markers of oxidative stress, mitochondrial function, inflammation, and apoptosis were examined. Tetrahydrocurcumin demonstrated significant neuroprotection on both hippocampal and dopaminergic neurons. The neuroprotection was attributed to its antioxidant, anti-apoptotic and mitochondrial marker-protecting actions. Furthermore, RNA sequencing was performed to validate the neuroprotective effects. Thus, Tetrahydrocurcumin can be an impactful therapeutic natural bioactive metabolite for preventing, reducing the rate of progression, and treating neurodegenerative pathologies.

Disclosures: S. Pathak: None. **K. Liu:** None. **M. Dhanasekaran:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR384.10/G10

Topic: C.03. Parkinson’s Disease

Support: Armenian National Science and Education Fund (23AN:NS-biochem-2925)..

Title: Electrophysiological and behavioral features of Neuroprotector Sutherlandia frutescens when Parkinson’s Disease

Authors: *N. BEHNAM DEHKORDI[1,2], V. SARKISIAN, Sr.[3];
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Abstract: Electrophysiological and behavioral features of Neuroprotector Sutherlandia frutescens when Parkinson’s Disease *** N. BEHNAM DEHKORDI**., V.SARKISIAN. **Ethnopharmacological relevance**: Sutherlandia frutescens L. (SF) is a plant used traditionally

in South Africa for various health conditions, including neurological disorders. Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra, resulting in motor symptoms. Rotenone, a pesticide, has been linked to PD-like symptoms in both in vitro and in vivo studies. However, specific effects of SF on PDrelated symptoms have not been extensively studied. **Aim of the study**: This study aimed to investigate the potential neuroprotective effects of SF against rotenone-induced PD using in vivo electrophysiological recordings from the hippocampus and an open field test to assess motor behavior. **Materials and methods**: Rats were divided into three groups: a control group receiving sunflower oil, a rotenone group receiving rotenone (2.0 mg/kg), and an SF group receiving hydroponically grown SF extract. Electrophysiological recordings from the hippocampus were conducted to assess neuronal activity, and an open field test was used to evaluate motor behavior. **Results**: Rats treated with SF exhibited significantly higher activity levels compared to both the sunflower oil and rotenone groups, indicating an activating effect of SF on motor behavior. Conversely, the rotenone group showed lower activity levels and exploration, suggesting a suppressive effect of rotenone on motor function. These results suggest that SF may modulate hippocampal neuron activity and potentially offer neuroprotective effects against rotenone-induced Parkinson's-like symptoms. **Conclusions**: SF, a plant with traditional medicinal use, shows promise in modulating motor behavior and hippocampal neuron activity in a rotenone-induced PD model. Further studies are warranted to elucidate the underlying mechanisms and clinical relevance of SF in PD management.
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Disclosures: N. Behnam Dehkordi: None. **V. Sarkisian:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

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Program #/Poster #: PSTR384.11/G11

Topic: C.03. Parkinson’s Disease

Support: NIH R01 NS115809
NIH F30 NS141487

Title: Estrogen-dependent endoplasmic reticulum-exit site upregulation protects against dopaminergic neuron loss in a mouse model of Parkinson’s Disease

Authors: *R. C. GARCIA[1], G. PANDEY[3], S. ZARATE[4], L. KUNAM[2], M. ZENEBE[2], R. SRINIVASAN[5];
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Abstract: Parkinson's disease (PD) is the second-most common neurodegenerative disorder globally and is pathologically characterized by a loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). Unmitigated endoplasmic reticulum (ER) stress, resulting from the accumulation of unfolded and misfolded proteins in the ER, plays a pivotal role in this loss of DA neurons and is a potential therapeutic target. Recent epidemiological studies have shown an inverse correlation between nicotine exposure and risk of developing PD, suggesting that nicotine exposure may be neuroprotective. Interestingly, cytisine, a smoking cessation drug and neuronal nicotinic acetylcholine receptor (nAChR) agonist, reduces ER stress and upregulates Sec24D-containing ER-exit site (ERES) formation in DA neurons by acting as a pharmacological chaperone of nAChRs. Furthermore, recent experiments have shown that in a 6-hydroxydopamine (6-OHDA) mouse model of PD, cytisine is neuroprotective in female mice and that such neuroprotection requires the presence of systemically circulating 17β-estradiol. Therefore, we hypothesize that upregulation of ERES in SNc DA neurons is critical for protection against ER stress-induced DA neuron loss, and that this upregulation is dependent on the presence of 17β-estradiol. To test this, we depleted estrogen via letrozole administration in cytisine-treated female mice and immunostained midbrain sections for the ERES marker Sec24D. We found that cytisine increased ERES formation in SNc DA neurons of female mice, however, the addition of letrozole inhibited this effect. Furthermore, in a transgenic mouse line with constitutively upregulated nAChRs, we found that only female mutant mice exhibit ERES upregulation and are protected against DA neuron loss in the 6-OHDA model. However, male mutant mice treated with brain-selective estrogen are protected against loss of SNc DA neurons and contain DA neuron ERES with significantly increased Sec24D protein content. Taken together, these data suggest that 17β-estradiol in conjunction with nAChR chaperoning play an important role in upregulating ERES, which has important implications in mitigation of DA neuron ER stress for the treatment of PD.

Disclosures: R.C. Garcia: None. **G. Pandey:** None. **S. Zarate:** None. **L. Kunam:** None. **M. Zenebe:** None. **R. Srinivasan:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR384.12/G12

Topic: C.03. Parkinson's Disease

Support: NOVOREX INC 144417

Title: Nrx02067: a selective cns-penetrant lrrk2 inhibitor with disease-modifying potential in parkinson's disease models

Authors: ***A. KHO**[1], E. LEE[1], E. KIM[2], H.-Y. HWANG[2], D. LEE[2], H. PARK[2], W. SON[2], H. KO[1];

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Abstract: The lack of disease-modifying therapies for Parkinson's disease (PD) highlights a major unmet medical need. Among the known genetic contributors, mutations in the leucine-rich repeat kinase 2 (LRRK2) gene represent the most frequent cause of inherited PD. Additionally, elevated LRRK2 kinase activity has been observed in sporadic cases, suggesting a broader relevance. This abnormal activity—arising from kinase hyperactivation and dysregulated scaffolding functions—has been linked to neuronal toxicity and neurodegeneration. As such, LRRK2 inhibition has emerged as a promising therapeutic strategy. Yet, earlier LRRK2 inhibitors have been hindered by limited selectivity, insufficient brain penetration, and safety concerns. To overcome these challenges, we developed **NRX02067**, a CNS-penetrant, highly selective, ATP-noncompetitive small-molecule LRRK2 inhibitor. In preclinical models, NRX02067 effectively suppressed LRRK2 kinase activity, as demonstrated by reduced phosphorylation of LRRK2 at Ser935 and its substrate Rab10 at Thr73. NRX02067 improved neuronal viability in primary cortical neurons expressing G2019S LRRK2 and restored lysosomal function in astrocytes derived from G2019S LRRK2 knock-in mice. Furthermore, in a cellular model of PD induced by α-synuclein preformed fibrils (PFF), NRX02067 rescued both neuronal toxicity and α-synuclein pathology. In a mouse model of α-synuclein PFF-induced PD, chronic NRX02067 treatment significantly improved behavioral performance (pole test, rotarod, grip strength), preserved dopaminergic neurons, reduced Lewy pathology, and suppressed neuroinflammation. Importantly, long-term treatment did not result in weight loss, reinforcing its safety for extended use. **These findings collectively position NRX02067 as a compelling candidate for disease modification in Parkinson's disease, offering both efficacy and long-term safety in preclinical models.**
Keywords: Parkinson's disease, LRRK2, ATP-noncompetitive small-molecule inhibitor, α-synuclein, Preformed fibrils (PFF), Dopaminergic neuronal loss, Neuroinflammation, Lysosomal dysfunction, Lewy bodies pathology

Disclosures: A. kho: None. **E. Lee:** None. **E. Kim:** None. **H. Hwang:** None. **D. Lee:** None. **H. Park:** None. **W. Son:** None. **H. Ko:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

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Program #/Poster #: PSTR384.13/G13

Topic: C.03. Parkinson's Disease

Support: CNPq[200336/2023-1]
Capes [88887.694614/2022-00]

Title: Evaluation of Nociception in a Model of Parkinson's Disease for the Study of Non-Motor Symptoms in Trpv4$^{-/-}$ mice

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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder of the central nervous system. The PD patients also suffer from non-motor symptoms, such as pain. Traditional PD models often present motor deficits, which can compromise the assessment of nociception. A role for the TRP4 receptor has been studied in the PD model. This study aimed to investigate the role of the TRPV4 receptor in regulating nociception experimental PD model for the study of non-motor symptoms. We used female and male mice C57BL/6 KO for TRPV4 and mice C57BL/6 wild type induced by 6-OHDA bilateral injection into the striatum. Behavioral analysis was performed at baseline and on the 7th, 14th, and 21st days after induction. The rotating cylinder test evaluated possible motor function and balance deficits. The mechanical allodynia was assessed using the Von Frey filament's up-and-down method. Heat allodynia was assessed using the heating plate test at a constant temperature of 38º degrees (CEUA protocol 3164260423).Euthanasia and collection of samples for PCR and immunohistochemistry occurred on the 21st day after induction. Our results did not demonstrate significant differences in forced locomotion between groups on the days evaluated after PD induction, indicating the absence of substantial locomotor damage. The peak of mechanical allodynia was different for males and females DP WT mice, 21 and 7 days after induction, respectively ($p<0.0001$). However, TRPV4 KO animals did not develop mechanical allodynia. Male and female DP WT mice exhibited differences in heat allodynia on the 14th, 21st and 28th days, with a peak of heat allodynia on the 14th day after induction compared to Sham WT animals ($p<0.001$). We did not observe heat allodynia in TRPV4 KO mice. We also observed increased expression of the TRPV4 receptor by PCR in PD WT mice. The tyrosine hydroxylase immunohistochemistry and the cylinder test confirmed the PD model. In conclusion, gene deletion of the TRPV4 receptor was shown to effectively protect the development of mechanical and heat allodynia in the bilateral 6-OHDA injection model. Behavioural assessment at different time points after induction provided insight into nociception. This study provides understanding for future research and paves the way for more refined approaches to learning and treating nociception in PD models.

Disclosures: L.G. Pereira: None. **G. Trevisan:** None. **S.Q. Kudsi:** None. **P. Rodrigues:** None.

Poster

PSTR384: Parkinson's Disease: Neuroprotective Mechanisms

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Program #/Poster #: PSTR384.14/G14

Topic: C.03. Parkinson’s Disease

Support: NIH INBRE Pilot Grant

Title: Testing the protective capacity of a small molecule against sporadic and genetic models of Parkinson’s disease in Drosophila

Authors: *A. C. ATHEBY[1], K. D. ROSIKON[2], H. O. LAWAL[1];
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Abstract: PD is the second most common neurodegenerative disease. Despite leaps in our understanding of the disorder following decades of research, the precise cause for the vast majority of cases remains unknown, there is still no known cure and viable treatments remain elusive. In this study, we are interested in developing neuroprotective strategies against the disease by testing the efficacy of the small molecule dacarbazine (which we identified in a previous pharmacological screen) and its structural derivative, 5-Amino-4-imidazolecarboxamide (AICA) using two model systems, rotenone an environmental toxin, and alpha synuclein, the first identified Parkinson's disease causal gene Rotenone is a potent laboratory model for sporadic PD that has been used to uncover important insights into the etiology of Parkinson's disease. Both dacarbazine and AICA have been reported previously to enhance synaptic activity in a manner that is dependent on vesicular monoamine release. In this project, we investigated whether both compounds are capable of conferring organismal and/or neuroprotection against rotenone toxicity. We report that dacarbazine confers a small but reproducible protection against organismal toxicity induced by rotenone exposure in both male and female Drosophila. . Moreover, we report preliminary findings that, AICA the dacarbazine structural analog protects dopamine (DA) neurons against rotenone-induced neuronal toxicity in an assay in which we combined both a pesticide (rotenone) and age as risk factors for PD. A focal point of our work is the testing of both compounds on genetic model of PD in which we generated a a myc::RFP tagged alpha synuclein transgenic line. Here we present the preliminary results the study to measure the effect of dacarbazine and AICA against defects induced by this α-synuclein overexpression. Taken together, these findings advance our understanding of a potentially viable strategy to intervene against Parkinson's disease.

Disclosures: A.C. Atheby: None. **K.D. Rosikon:** None. **H.O. Lawal:** None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.01/G15

Topic: C.06. Neuromuscular Diseases

Support: NINDS Intramural Research Program

Title: Evaluating the utility, validity, and responsiveness of the adverse event unit for congenital myasthenic syndromes

Authors: *R. PATEL[1], M. HEHIR[3], M. MCANALLY[2], M. GOODEN[1], S. MARTIN[1], A. POTTICARY[2], M. CONAWAY[4], L. REOMA[1];
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Burlington, VT; [4]Dept. of Publ. Hlth. Sciences, Div. of Translational Res. and Applied Statistics, Univ. of Virginia, Charlottesville, VA

Abstract: Adverse events (AEs) and their associated burden largely impact patient treatment adherence and choices. The Adverse Event Unit (AEU) is a patient- and physician-weighted consensus unit that quantifies and compares AE burden among any class of medications for neurologic conditions. Pilot studies have demonstrated preliminary feasibility and validity of the AEU in adult patients with myasthenia gravis. However, whether the AEU can inform treatment tolerability for patients with congenital myasthenic syndromes (CMS), a group of inherited disorders of the neuromuscular junction, remains to be investigated. The purpose of this study is to assess the utility, content validity, and responsiveness of the AEU as a measure of comparative AE burden in a natural history study of pediatric and adult patients with CMS. It is hypothesized that the AEU will strongly correlate with changes in CMS treatment type and dosage and weakly correlate with standard quality of life metrics. The AEU will be administered to up to 75 patients aged at least 6 months with clinically stable CMS (e.g., DOK7 and COLQ variants confirmed via genetic testing and clinical history) enrolled in an NIH natural history study. The AEU metric entails a survey that weighs the severity of AEs associated with standard CMS treatments. AEU patient responses will be collected via a standard method by a trained clinical evaluator biannually. Caregivers will provide responses for patients under 10 years. Reports of adverse and disease-related events will be continuously collected throughout the study. A literature review of AE reports was conducted to determine which AEs to include in study administration of the AEU. A total of 50 AEs were identified as potentially relevant for patients treated with standard of care therapies for CMS. Of these 50 AEs, 33 (66%) were coded in the current development of the AEU, whereas 17 (34%) were not. These preliminary results suggest the AEU may not capture all the potential side effects patients with CMS may experience. However, the literature review revealed a gap in AE data reporting in CMS clinical trials. Thus, AE data collected from this natural history protocol may better establish content validity of the AEU and identify additional AEs to be included in the AEU. As side-effect burden informs patient experience, improving the methodology of measuring AE burden is critical in clinical research. Investigating the application of the AEU in patients with CMS may refine this tool for future adeno-associated virus gene therapy trials. Furthermore, this study can inform the methodology and framework of future validation studies of the AEU with various patient populations.

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Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

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Topic: C.06. Neuromuscular Diseases

Support: NIH Grant AI093504
NIH Grant AI173059
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DTRA BAA CB11178
DOD OTA MCDC2212-003

Title: Development of new aminopyridines for the safe and effective treatment of peripheral neurological disorders

Authors: *P. M. MCNUTT;
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Abstract: Aminopyridine-based voltage-gated potassium channel (VGKC) blockers, such as 3,4-diaminopyridine (3,4-DAP) and 4-aminopyridine, enhance neurotransmitter release by prolonging presynaptic action potentials. The FDA-approved compound 3,4-DAP has demonstrated symptomatic efficacy in the treatment of various peripheral neuromuscular disorders involving reduced presynaptic release, including Lambert-Eaton myasthenic syndrome, myasthenia gravis, and congenital myasthenic syndromes. More recently, 3,4-DAP has shown promise as a fast-acting reversal agent for botulinum neurotoxin (BoNT)-induced paralysis, a condition marked by impaired acetylcholine release due to neuronal SNARE protein cleavage. However, widescale use of 3,4-DAP is limited by two properties: a narrow therapeutic index due to dose-limiting CNS toxicities and five-fold interindividual variability in plasma exposure driven by NAT2 polymorphisms. To address these limitations, we developed a new class of aminopyridine analogs designed to retain VGKC blocking activity while reducing CNS penetration and eliminating NAT2 metabolism. Guided by a structure-based pharmacophore model incorporating a protonatable ring nitrogen and hydrogen bond donor at the 4-position and informed by Alphafold predictions of molecular binding interactions, we synthesized analogs with targeted substitutions to modulate activity, permeability, and metabolic stability. Lead compounds were screened in a BoNT/A mouse model using whole-body plethysmography; multiple candidates restored respiratory function to normal physiological levels at well-tolerated doses. Pharmacokinetic studies revealed extended half-lives, favorable bioavailability, and resistance to acetylation in human liver S9 fractions. Ex vivo nerve-muscle preparations and ion channel recordings confirmed potent inhibition of VGKCs associated with motor neuron repolarization and presynaptic ACh release. Lead compounds are advancing into translational development as fast-acting NAT2-resistant therapeutics for BoNT intoxication and other peripheral neuromuscular pathologies. These findings validate an aminopyridine drug discovery platform with the potential to identify VGKC modulators targeting new indications, such as pain, muscular dystrophy, and seizures.

Disclosures: P.M. McNutt: None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

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Topic: C.06. Neuromuscular Diseases

Support: NIAID Grant AI093504
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DTRA Grant: HDTRA1-22-1-0029
DTRA Grant: MCDC2201-002

Title: A Human Pluripotent Stem Cell-Derived Neuromuscular Junction Model Reveals Botulinum Neurotoxin A-Induced Synaptic Dysfunction

Authors: *C. A. AGUAYO, P. M. MCNUTT;
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Abstract: The human neuromuscular junction (NMJ) is a specialized synapse that modulates neurological control over muscle contraction While the neurophysiological properties of the NMJ have been extensively characterized in animal models, our knowledge of human NMJ neurophysiology remains limited. Compared to animal models, human NMJs are smaller, less complex, and more fragmented, with divergent molecular and cellular characteristics. Furthermore, NMJs are vulnerable entry points for pathogens and toxins that can impair motor function and propagate central nervous system infections. Despite the clinical importance of NMJ's, existing methods for studying human NMJs remain limited, hindering our ability to model disease mechanisms and develop targeted therapies. We optimized a protocol for generating functional human NMJs through directed differentiation of pluripotent stem cells via a neuromesodermal pathway, yielding cultures that recapitulate key features of native human NMJs, including tripartite cellular composition, molecular organization, synaptic architecture, and electrophysiological properties. Quantitative PCR analysis of gene expression at days 6, 15, and 25 confirmed progressive NMJ maturation through expression of neuronal (TUBB3, ChAT), muscle (CHRNA1, FBXO32), and synaptic (SV2) markers, indicating functional neuromuscular connectivity. NMJ formation was further validated by using α-bungarotoxin to label nicotinic acetylcholine receptors, and immunocytochemistry to detect mature neuronal cells (neurofilament) and skeletal muscle (myosin) markers. To validate this platform as a tool for investigating neurotoxin-induced pathologies, we characterized the effects of two neuroparalytic toxins. First, exposure to the neuromuscular blocking agent curare led to total inhibition of contraction within 5 min (n=4), by blocking nicotinic acetylcholine receptors and preventing neural activation of skeletal muscle. Second, we exposed the NMJ's to botulinum neurotoxin while evaluating synaptic transmission using multielectrode arrays. BoNT/A exposure at 10 pM (n=4) significantly decreased mean firing rate by 80% and burst frequency by 90% compared to vehicle-treated controls (n=4, $p<0.001$, two-way ANOVA followed by Dunnett's multiple comparison test), while culture viability remained unchanged. These results demonstrate that the platform expresses the key features of neuromuscular transmission. This validated human model enables mechanistic investigation of neurotoxin-induced paralysis and provides a translational platform for developing countermeasures against NMJ toxicity.

Disclosures: C.A. Aguayo: None. **P.M. McNutt:** None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

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Program #/Poster #: PSTR385.04/H2

Topic: C.06. Neuromuscular Diseases

Support: NIH AI093504
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NIH AI180532

Title: Modeling neurological Chikungunya virus infection in human iPSC-derived midbrain organoids reveals extracellular vesicle-mediated viral packaging

Authors: J. A. CLEARY[1], ***T. G. WENTZ**[1], P. M. MCNUTT[2];
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Abstract: Chikungunya virus (CHIKV) is a neuroinvasive alphavirus capable of causing severe neurological sequelae. Despite increasing clinical reports of neuroinfection, the mechanisms underlying CHIKV neuropathogenesis remain poorly understood. As a biosafety level 2 (BSL-2) pathogen, CHIKV also offers a tractable experimental model for investigating neuroinvasion pathways shared with more highly restricted BSL-3 alphaviruses, such as Eastern and Venezuelan equine encephalitis viruses. Here, we use human iPSC-derived midbrain organoids to model CHIKV infection and reveal key features of viral neuropathogenesis. CHIKV productively infects multiple neural cell types, leading to widespread cell death and the production of infectious progeny within 72 hours. Transcriptomic profiling shows that CHIKV infection acutely suppresses host innate immune responses, suggesting active modulation of antiviral defense pathways. Strikingly, we find that CHIKV genomes are actively packaged into extracellular vesicles (EVs) derived from infected organoids, including both medium-to-large (mlEVs) and small EVs (sEVs). Super-resolution microscopy and transmission electron microscopy with immunogold labeling reveal that subsets of these vesicles display the CHIKV E1 and E2 spike proteins. Immunoaffinity capture further demonstrates that viral RNA is enriched in EV populations that are positive for CD63, E1, or both ($CD63^+/E1^+$). These data suggest that CHIKV selectively co-opts EV biogenesis pathways to package and possibly shield viral components during neuroinvasion. Ongoing studies with Oropouche and Powassan viruses will test whether the incorporation of viral proteins into host-derived EVs represents a conserved strategy among neuroinvasive RNA arboviruses. Together, these findings establish human midbrain organoids as a powerful platform to dissect EV-mediated responses to viral infection and to identify host-virus interactions that may serve as novel targets for therapeutic intervention.

Disclosures: J.A. Cleary: None. **T.G. Wentz:** None. **P.M. McNutt:** None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

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Program #/Poster #: PSTR385.05/H3

Topic: C.06. Neuromuscular Diseases

Support: Maryada Foundation

Title: Uncovering Noncanonical Glial Responses to Different Structural and Compositional Variants of Botulinum Neurotoxin Using 3D Culture and Transcriptomic Profiling

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Abstract: Botulinum neurotoxin A (BoNT/A) is widely recognized for its ability to cleave SNAP-25 and block neurotransmitter release. Emerging evidence suggests BoNT/A can influence central nervous system signaling through SNAP-25-independent mechanisms. In this study, we developed a 3D glial culture model using primary neurons, astrocytes, and microglia. The cultures were treated with three structurally distinct BoNT/A forms: the purified holotoxin; the progenitor complex, which includes non-toxic associated proteins (NAPs); a catalytically inactive derivative (Dr-BoNT) produced through recombinant technology; and NAPs alone. Live-cell calcium imaging showed form-specific reductions in intracellular calcium influx: 24.65% ± 7.20 (holotoxin), 13.00% ± 9.74 (complex), 10.56% ± 7.43 (Dr-BoNT), and 1.38% ±6.22 (NAPs), suggesting SNAP-25-independent modulation of neurotransmission. Cytokine profiling indicates glial activation via altered expression of IL-6, IL-8, IFN-γ, and IP10 independent of SNAP-25 cleavage. Transcriptomic profiling via microarray was performed on SH-SY5Y cells treated with the holotoxin, NAPs and complex revealing distinct early (4 h) and late (48 h) gene expression responses. At 4 h, DEGs were highest with holotoxin (1,873), followed by complex (752) and NAPs (458). At 48 h, DEGs shifted: NAPs (3,176), complex (2,129) and holotoxin (705), highlighting form- and time-dependent activation of divergent downstream signaling pathways. These findings show that BoNT/A can modulate glial physiology and immune responses through noncanonical mechanisms unrelated to its enzymatic activity. Our data also suggests that active and inactive forms of BoNT/A engage distinctive cellular pathways, highlighting a broader immunomodulatory potential beyond motor neuron blockade. We also demonstrate the importance of 3D culture systems for capturing complex glial responses to neurotoxins.

Disclosures: G. Ambrin: None. **L. Wang:** None. **A. Yousuf:** None. **B. Singh:** None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

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Program #/Poster #: PSTR385.06/H4

Topic: C.06. Neuromuscular Diseases

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National Research Foundation of Korea(NRF) grant funded by the Korea government (MSIT) RS-2024-00509145

Title: Schwann cell-derived extracellular vesicles promote neuromuscular regeneration through intercellular communication

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Abstract: Neuromuscular junction (NMJ) is a critical target for treating age-related neuromuscular disorders. Chronic inflammation in aging impairs the repair ability of the peripheral nervous system, inhibiting Wallerian degeneration/regeneration of presynaptic structures and leading to neuromuscular dysfunction. Although Schwann cells have been suggested as key players in neuronal regeneration, their precise inflammatory response mechanisms remain poorly understood. To identify novel therapeutic targets for NMJ-related disorders, we investigated the role of Schwann cells in the NMJ and muscle regeneration under inflammatory conditions.To examine Schwann cell morphology in NMJ regeneration, we treated mice with cardiotoxin and LPS to induce tissue repair and inflammation. We observed transverse abdominal and lumbrical muscles of young (4m) and old mice (22-30m) via immunohistochemistry using NMJ markers. We found that NMJ defects accompanied by abnormal Schwann cells in both aged and inflammation-induced mice, suggesting Schwann cell involvement in NMJ abnormalities. Remarkably, while normal Schwann cells exhibited dynamic changes from injury to regeneration, inflammation-exposed Schwann cells lost this activity, indicating impaired molecular functions in the regenerative environment. Given the NMJ's unique multicellular composition, we hypothesized that Schwann cells would communicate through specific mediators, particularly extracellular vesicles (EVs), which might serve as regenerative mediators. Based on our experimental hypothesis that Schwann cells enhance regeneration through EVs, we isolated EVs from S16 Schwann cell lines to analyze their functional properties. The isolated EVs were characterized using nanoparticle trafficking analysis, and western blotting. Comparison of EVs from inflamed and non-inflamed Schwann cells revealed differences in size, number, and protein content. Furthermore, we performed a proteomic analysis of Schwann cell-derived EVs and identified a cluster of proteins involved in both neuron development and muscle growth factor pathways, including those regulating early myoblast differentiation. To validate the functional role of these proteins, we treated damaged C2C12 myoblast cells with Schwann cell-derived EVs and demonstrated their ability to accelerate myoblast differentiation. These findings reveal a novel mechanism whereby Schwann cells regulate NMJ maintenance and muscle regeneration through EVs containing specific proteins, providing insight into potential therapeutic strategies for age- and inflammation-related NMJ dysfunction.

Disclosures: H. Jeong: None. **K. Lee:** None. **J. Lee:** None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.07/H5

Topic: C.06. Neuromuscular Diseases

Title: N-acetylcysteine reverses alterations in redox status, expression of myogenic miRNAs and muscle damage induced by subchronic administration of cisplatin in female rats

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Abstract: Cisplatin (CIS) is a potent chemotherapeutic widely used, but its administration is frequently associated with adverse effects such as muscle atrophy and functional weakness. While recent evidence suggests that N-acetylcysteine (NAC), a well-known antioxidant, can mitigate CIS-induced neurocognitive and muscular impairments, its influence on the expression of myogenic microRNAs (miRNAs), key regulators of muscle differentiation and regeneration, remains poorly understood. This study aimed to evaluate the protective effects of NAC on redox balance, myogenic miRNA expression, and skeletal muscle structure and function in a rat model of subchronic CIS administration. Adult female Wistar rats were divided into four groups: Control, NAC (300 mg/day for 8 days), CIS (3 mg/kg i.p. for 5 days), and NAC+CIS (NAC for 8 days, with CIS administered from day 4). Five days after the last CIS dose, grip strength was measured, and muscle tissue was analyzed for GSH/GSSG ratio, expression of miR-1-3p, miR-133a-3p, miR-206-3p, and histological alterations. CIS-treated rats exhibited reduced grip time and a trend toward decreased muscle strength, accompanied by a lower GSH/GSSG ratio and downregulation of miR-1-3p, with tendencies toward decreased miR-133a-3p and miR-206-3p. Morphological abnormalities in muscle fibers were also observed. Notably, coadministration of NAC restored muscle strength, normalized the redox status, reversed the downregulation of

myogenic miRNAs, and attenuated muscle fiber damage. These findings indicate that NAC counteracts CIS-induced muscular toxicity by improving redox balance and regulating miRNA expression, supporting its potential as an adjuvant therapy to prevent chemotherapy-associated muscle damage.

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Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.08/H6

Topic: C.06. Neuromuscular Diseases

Title: A translational and quantitative modeling framework for TfR-mediated delivery of gene silencing therapies for myotonic dystrophy type 1

Authors: *M. PABLO[1], M. NISHIHARA[2], Y. MUGURUMA[2], N. ZACH[1], T. YONEYAMA[1]; [1]Takeda Develop. Ctr. Americas, Inc., Cambridge, MA; [2]Takeda Pharmaceut. Co. Ltd., Kanagawa, Japan

Abstract: Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disease caused by an abnormal CTG repeat expansion in the *DMPK* gene. Transcribed DMPK with this expansion forms RNA foci that sequester splicing regulators leading to aberrant splicing and ultimately DM1 disease manifestations. In the clinic, oligonucleotide-based gene silencing of DMPK has been attempted, and challenges in drug delivery to key tissues has led to the application of Transferrin Receptor (TfR)-mediated delivery approaches [1-3]. Next-generation therapies for DM1 can learn from existing preclinical and clinical PK/PD data. We developed a modeling framework using public data from two molecules under clinical investigation for DM1 treatment [2-3]. We used our framework to analyze PK/PD in nonhuman primates (NHPs) and its translatability to DM1 patients, including the effect of DMPK reduction on the Splice Index (SI), which captures associations between missplicing and physical strength/mobility.
A mechanistic model for TfR-mediated delivery to muscle was developed, accounting for both systemic and muscle TfR expression. Drug exposure in muscle was linked to DMPK knockdown via an indirect response model. The model was fit to PK/PD data from NHPs dosed with AOC-1001, a DMPK-targeting siRNA conjugated to an anti-TfR mAb currently in clinical development. Parameters were translationally adjusted using physiological values and allometric scaling, and the model was used to predict PK/PD in DM1 patients participating in the AOC-1001 Phase 1/2 trial (MARINA). The same framework was applied in a reverse-translational manner, by calibrating to PK/PD data from patients receiving DYNE-101, a DMPK-targeting

ASO conjugated to a Fab, in a Phase 1/2 trial (ACHIEVE), then predicting available NHP PD response. The effect of DMPK knockdown on SI over time was modeled using data from MARINA, ACHIEVE, and the END-DM1 natural history study [4].
The model reasonably fit the available data, and our scaling approach predicted PK well. Assuming equivalent PD parameters between NHP and human for AOC-1001 overpredicted DMPK knockdown, which was empirically corrected by adjusting the maximal knockdown. For DYNE-101, the same PD parameters could be applied across NHP and human. The estimated effect of DMPK reduction on SI was consistent across the two molecules. This is a useful tool to guide the development of next-generation oligonucleotide therapies for DM1 patients.
[1] Thornton et al. Lancet Neurol 2023, 22: 218-28 [2] www.aviditybiosciences.com/platform/publications [3] www.dyne-tx.com/our-forcetm-publications [4] Provenzano et al. J Clin Invest 2025, 135(4):e185426

Disclosures: M. Pablo: A. Employment/Salary (full or part-time): Takeda. **M. Nishihara:** A. Employment/Salary (full or part-time): Takeda. **Y. Muguruma:** A. Employment/Salary (full or part-time): Takeda. **N. Zach:** A. Employment/Salary (full or part-time): Takeda. **T. Yoneyama:** A. Employment/Salary (full or part-time): Takeda.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.09/H7

Topic: C.06. Neuromuscular Diseases

Title: Corticosteroid Deflazacort Impairs Social Behavior in MDX Mice

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Abstract: Rational and Objectives: Duchenne muscular dystrophy (DMD) is a rare genetic disease, resulting in irreversible degeneration of the skeletal, smooth and cardiac muscles due to the loss of a cytoskeletal protein called dystrophin. From the age of 35, patients die of heart or respiratory failure. In the absence of a cure, only palliative corticotherapy treatment is currently used in DMD patients. Unfortunately, corticoids such as prednisone and deflazacort (DF) are associated with modest benefits but also with severe side effects especially with prolonged use[1]. This may include weight gain, bone decalcification, and DF is also suspected to induce changes in emotional and social behaviors (depression, irritability, anxiety). However, whether corticoids worsen these features is poorly understood. Currently, numerous studies have described that mouse models of DMD display similar feature as DMD patients but the knowledge on the detrimental effects of DF on social behavior in a mouse model of DMD is lacking. Here, in our

study we used the mdx mouse model, to test whether DF treatment has an impact on the social behavior alterations seen in mdx mice.
Methods:*Animals*: male mdx-B6 mice were subcutaneously injected with DF (1mg/kg/day, 3 times/week for 5 weeks) and their WT littermates with a saline solution.*Social behavior tests*: Standard protocols included anxiety tests (Dark- Light choice, Openfield exploration and Elevated plus-maze test), tests assessing unconditioned fear (restraint-induced fear) and social behavior using social interaction tests and social communication analyses (ultrasonic vocalizations during direct social interaction).
Results: Our data bring evidences that DF treatment is detrimental for social behavior. Indeed, we found that DF did not worsen the anxiety trait seen in mdx mice but worsened the ability of mdx mice to establish new contacts with a mouse visitor. Additionally, important behaviors such as dominance, pursuits, types and number of contacts or immobility were altered.**Conclusion:** Our results suggest that DF may reduce the social ability of a mouse to interact with a fellow conspecific. Given the widespread use of corticosteroid therapy and its detrimental effect on social behavior, our findings regarding DF call for a more rigorous evaluation of its role on patients' social behavior.
1. Matthews, E., Brassington, R., Kuntzer, T., Jichi, F. & Manzur, A. Y. Corticosteroids for the treatment of Duchenne muscular dystrophy. Cochrane Database of Systematic Reviews (2016) doi:10.1002/14651858.CD003725.pub4.

Disclosures: J.I. Bikouta: None. **L. Ceschi:** None. **C. Vaillend:** None. **S. de la PORTE:** None. **J. Cancela:** None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

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Program #/Poster #: PSTR385.10/H8

Topic: C.06. Neuromuscular Diseases

Support: P20GM103451

Title: Proteomic and functional characterization of Lamin mutations in a Drosophila models of Muscular Dystrophy and Lipodystrophy

Authors: J. BAKER, *D. DONLEY;
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Abstract: Nuclear lamins are specialized intermediate filament proteins that form the nuclear periphery, providing structural support and regulating gene expression and translation. Mutations in the *LMNA* gene, which encodes lamin A and C proteins, cause laminopathies—a group of disorders including muscular dystrophy and lipodystrophy. Disruption of *LMNA* function compromises its protein products' roles as transcriptional regulators, signaling agents, and structural components of the nucleus. Although laminopathies arise from molecular defects in a

common gene, they result in highly diverse phenotypic outcomes. To investigate the tissue-specific effects of *LMNA* mutations, we conducted proteomic analyses in *Drosophila melanogaster* models expressing mutations in the *lamin C* gene corresponding to human muscular dystrophy and lipodystrophy variants. We report a comparative analysis of these Drosophila models, examining both mutant lines and wild-type controls. Differential protein expression was assessed in skeletal muscle and central nervous system (CNS) tissues. Herein, we report on distinct molecular signatures across genotypes and tissues, identifying protein expression changes unique to each laminopathy model. These findings suggest that subtle differences in mutation type or tissue context can drive divergent pathological effects. Prior studies indicate that both muscular dystrophy and lipodystrophy impair neuromuscular junctions, leading to motor control deficits and behavioral disruption. Our study highlights specific proteomic alterations that may underlie these impairments and offers insight into the shared and distinct molecular mechanisms by which laminopathies affect both neural and muscular systems.

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Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR385.11/H9

Topic: C.06. Neuromuscular Diseases

Title: Using orthogonal structural analyses to evaluate variants of unknown significance involved in mitofusin-2

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Abstract: Charcot-Marie-Tooth 2A (CMT2A) is an autosomal dominant neurological disorder characterized by axonal neuropathy and is implicated by MFN2 gene mutations, encoding for mitofusin-2 (MFN2). MFN2 is a dynamin-like GTPase involving the outer mitochondrial membrane, modulating mitochondrial tethering to the endoplasmic reticulum. However, the biological effects of missense mutations on MFN2 vary widely among phenotypes, with previous links to Alzheimer's disease, Parkinson's disease, and diabetes [1]. Despite its biophysical significance, not all variants of MFN2 have been classified (benign, gain-of-function, risk-inducing, or pathogenic), which is essential in defining their role in pathogenesis. Herein, we sought to define the effects of 9 variants of unknown significance (VUS): 4 on the GTPase domain (T236M, S249C, R280H, P251R) [2], 3 on the coiled-coil heptad-repeat (H361Y, R364P, R364W) [2], and 2 on the cytosolic heptad-repeat domain (W740S, H750S) [3], respectively. Using a previously validated protocol [4], we evaluated the functional, structural,

and stability effects on VUS of MFN2. We also performed our analyses on 3 commonly found pathogenic variants on Arg94 (R94Q, R94W, R94G) in patients with CMT2A as controls. Using the resolved protein structure of MFN2 (PDB: 6JFL), structural analyses were first performed. It was found that steric hindrance (local clashes) increased among all variants except for R364P and W740S, which decreased. No general trend was observed from the mutations from the relative solvent accessibility of the replaced residue. However, it was noted that P251R had significantly altered the structure as a charge residue was replaced and the clash scores had largely increased (+21.15%), and the R94W variant led to damage as an expansion of the cavity (70.632 $Å^3$), respectively. Next, we evaluated the predicted pathogenicity of the VUS and found that almost all variants were labeled as pathogenic, except for discrepancies with T236M and R364W as benign from AlphaMissense. Similarly, PolyPhen-2 had identified W740S and H750P as benign. Next, we performed stability analysis, changing the protocol to include DDGEmb [5] instead of INPS-MD and DeepDDG. From all VUS, it was found that S249C significantly destabilized the structure by -0.6330 ± 0.3599 kcal/mol ($p = 0.0171$), R280H by -0.9554 ± 0.6885 kcal/mol ($p = 0.0361$), and P251R by -1.0747 ± 0.5580 kcal/mol ($p = 0.0126$). All remaining variants did not lead to significant effects. Overall, from the orthogonal structural analysis performed in this study, we provide mechanistic insights into the critical VUS of MFN2 that may be informative to clinical efforts in the near future.

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Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

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Program #/Poster #: PSTR385.12/H10

Topic: C.06. Neuromuscular Diseases

Title: Tubular aggregates in patients with mitochondrial disease: a case series

Authors: *J. HU;
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Abstract: Tubular aggregates are unusual membranous structures in skeletal muscle and are often associated with genetic defects. While the phenomena have been observed in various myopathies, particularly tubular aggregate myopathy, their connection to mitochondrial disease has remained elusive. Here we present three patients diagnosed with mitochondrial disease, while the muscle biopsy showed tubular aggregates in addition to the expected mitochondrial metabolic abnormality pathology. The study included patients who were diagnosed with mitochondrial disease via biceps muscle biopsy at Peking University First Hospital from 2016 to 2024. Clinical data were retrospectively analyzed. Mitochondrial genetic testing was performed in two cases. Two females (aged 31 and 32 years) presented with chronic, progressive, asymmetric ptosis, with disease durations of 10 and 6 years, respectively. Mitochondrial genetic

analysis in one patient revealed a large-scale deletion (chrM:10166-13021). Muscle biopsies in both female patients demonstrated characteristic pathological features of mitochondrial myopathy, including ragged-red fibers (RRF), ragged-blue fibers (RBF), and cytochrome c oxidase (COX)-negative fibers. Additionally, modified Gomori trichrome staining revealed tubular aggregates in some muscle fibers. The third patient, a 21-year-old male, presented with recurrent seizures and psychiatric/behavioral abnormalities for over 1 year. Genetic testing identified a pathogenic MT-ND1 mutation (m.3946G>A). His muscle biopsy showed numerous subsarcolemmal and cytoplasmic tubular aggregates highlighted by NADH-TR staining, accompanied by occasional RRF, RBF, and subsarcolemmal mitochondrial accumulations. Notably, none of the three patients exhibited significant episodic muscle cramps, myalgia, myotonia, or limb muscle weakness. By describing these cases, we expand the phenotypic spectrum of tubular aggregates beyond tubular aggregate myopathy to include other multisystem disorders, such as mitochondrial disease. Although the underlying mechanisms remain poorly understood, our report could provide new insight into the role of mitochondria in the development of tubular aggregates.

Disclosures: J. Hu: None.

Poster

PSTR385: Disorders of Neuromuscular Junction and Muscle

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Title: Nicotinamide mononucleotide administration improves age-related neuromuscular junction denervation by reverting laminin β2 levels

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Abstract: Aging causes a decrease in neuromuscular function and innervation of the neuromuscular junction. There is a knowledge gap in the primary mechanism causing age-related neuromuscular junction denervation. This mechanism was investigated by focusing on the synapse organizer laminin β2, which is concentrated specifically at the synaptic cleft of this synapse. A loss of laminin β2 is known to cause degeneration of the neuromuscular junction in humans and mice. In this study, the expression level of laminin β2 at neuromuscular junctions

was detected using immunohistochemistry and compared between adult and aged mice. The necessity of laminin β2 signaling for neuromuscular junction maintenance was tested in wild-type mice by inhibiting the interaction of laminin β2 and its specific receptor. Aged mouse muscles exhibit reduced mitochondrial function; therefore, we treated aged mice with nicotinamide mononucleotide (NMN), which is known to improve tissue mitochondrial function. NMN-administered aged mice were assessed for muscle mitochondrial function, laminin β2 expression levels, neuromuscular junction innervation rates, and behavioral tests, compared to age- and sex-matched sham-treated mice. In addition, NMN was administered to cultured myotubes to test the direct signaling of NMN to muscle fibers for controlling laminin β2 expression. As a result, we identified an age-related decrease in synapse organizer laminin β2 at neuromuscular junctions. Chronic inhibition of laminin β2 signaling significantly increased neuromuscular junction denervation, suggesting that laminin β2 is necessary for maintaining neuromuscular junction innervation. Importantly, NMN administration ameliorated age-related neuromuscular junction denervation and increased the expression levels of laminin β2 along with its signaling proteins at motor nerve terminals. Extended NMN administration improved the moving speed and grip strength in aged mice. In vitro, NMN increased cytosolic ATP concentration and laminin β2 secretion level of cultured C2C12 myotubes. The results suggested that age-related neuromuscular junction denervation is caused by a reduction of synapse organizer laminin β2 at this synapse. NMN signaled directly to muscle cells and increased laminin β2 secretion, which improved neuromuscular junction innervation in aged mice. This study showed a potential intervention method that ameliorates the mechanism of age-related neuromuscular junction denervation.

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Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR386.01/H12

Topic: D.02. Ischemia

Support: NIH Grant R21NS141704

Title: Ccl2/ccr2 axis in hiv-1-associated risk of ischemic stroke

Authors: *S. TORICES[1], N. FATTAKHOV[2], S. DEL SOL[2], E. LARA[2], M. TOBOREK[3]; [1]Biochem., Univ. of Miami, MIAMI, FL; [2]Dept. of Biochem. and Mol. Biol., Univ. of Miami, Miami, FL; [3]Dept. of Biochem. and Mol. Biol., Univ. of Miami Sch. of Med., Miami, FL

Abstract: HIV infection increases the risk and severity of ischemic stroke by amplifying brain injury and impairing recovery. CCL2, a potent chemoattractant, plays a central role in recruiting CD4+ T cells and monocytes, thereby facilitating HIV-1 entry and persistence within the central nervous system. Additionally, CCL2 contributes to inflammatory processes and promotes disruption of the blood-brain barrier (BBB) during ischemic events. However, the specific role of CCL2 in the context of HIV-associated ischemic stroke remains poorly understood. To address this problem, we performed *in vitro* studies using co-cultures of human astrocytes and brain endothelial cells subjected to oxygen/glucose deprivation followed by reoxygenation (OGD/R), modeling stroke conditions. The obtained data demonstrated that astrocytes are the primary producers of CCL2 in the CNS. Importantly, treatment with cenicriviroc (CVC), a dual CCR2/CCR5 antagonist, inhibited NLRP3 inflammasome activation in human astrocytes exposed to both OGD/R and HIV-1, suggesting a role for CCR2 signaling in HIV-related neuroinflammation. Moreover, C57BL/6 mice were infected with EcoHIV, and ischemic stroke was induced via the middle cerebral artery occlusion (MCAO) technique. EcoHIV-infected mice subjected to ischemic stroke significantly increased levels of CCL2 and glial fibrillary acidic protein (GFAP), indicating elevated astrocyte reactivity. HIV infection also enhanced susceptibility to ischemic strokes. Overall, these findings support the hypothesis that CCL2/CCR2 signaling contributes to worsened ischemic stroke outcomes in the context of HIV-1 infection. This pathway may represent a promising therapeutic target to reduce neuroinflammation and improve post-stroke recovery in people living with HIV.

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Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.02/

Topic: D.02. Ischemia

Support: R01NS115835

Title: Identification and Characterization of SINE-RNA Expression in the Brain following Ischemic Stroke

Authors: *D. SATAV[1,2], S. LIMAM[1,2], A. DHARAP[1,2];
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Abstract: Short interspersed nuclear elements (SINEs) are a highly abundant class of retrotransposable elements that can be transcribed by RNA polymerase III into long noncoding RNAs (SINE-RNAs). Previous studies have shown that SINE-RNAs are induced under cellular stress conditions such as heat shock and viral infection, where they contribute to chromatin

remodeling, transcriptional repression, nuclear retention of mRNAs, and activity-dependent gene regulation. However, their role in ischemic stroke remains largely unexplored. This study aimed to identify and characterize stroke-induced SINE-RNAs in a C57BL/6N mouse model of transient focal cerebral ischemia. Male mice were subjected to 1 h of middle cerebral artery occlusion followed by 6 h, 12 h, or 24 h of reperfusion (MCAO/R). Deep RNA-sequencing (n = 3 per group) of the ipsilateral sensorimotor cortex was performed to identify differentially expressed transcripts. Novel transcripts were filtered *in silico* based on the presence of SINE elements near the 5′ end with conserved A-box and B-box motifs and termination near canonical RNA Pol III termination sequences (TTTT). We identified 335 stroke-induced SINE-RNAs at 6 h post-reperfusion, which represented the peak expression timepoint as compared to sham controls. Many of these transcripts remained induced through later timepoints during the acute reperfusion phase (24h). We performed single-molecule RNA in situ hybridization combined with immunohistochemistry using cell-type-specific markers (NeuN for neurons, GFAP for astrocytes, and Iba1 for microglia) to investigate their spatial and cellular distribution. We observed heterogeneous neuronal expression of SINE-RNAs throughout the ipsilateral cortex and hippocampus. We further evaluated their expression in an *in vitro* model using HT22 neuronal cells exposed to 8 h of oxygen-glucose deprivation followed by various reoxygenation timepoints (up to 24 h). Peak expression was observed at 9 h of reoxygenation, reflecting a similar induction pattern to that seen *in vivo*. Together, these results demonstrate that SINE-RNAs are dynamically and selectively expressed in neurons following ischemic injury both *in vivo* and *in vitro*. This work provides foundational insights into their spatial and cell-type specificity and lays the groundwork for functional studies. Future work will include knockdown of specific SINE-RNAs to assess their role in post-stroke pathology, and extension of this research to female mice to investigate sex-dependent responses.

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Poster

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Title: BRD4 activates TREM1-mediated neuroinflammation leading to neuronal death in the hippocampal CA1 following global cerebral ischemia

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Abstract: Global cerebral ischemia most commonly occurs due to cardiac arrest, causing selective and delayed death of hippocampal CA1 pyramidal neurons, which leads to severe cognitive deficits. Although global ischemia-induced neuronal death in hippocampal CA1 is well-documented, the underlying molecular mechanisms of neuronal death and the associated cognitive deficits are still unclear. To address this, we performed RNA-seq on the hippocampal CA1 at 24 and 48h after global ischemia to identify key regulators involved in delayed neuronal death following global ischemia. Ingenuity Pathway Analysis (IPA) of differentially expressed genes (DEGs) identified inflammatory response-related pathways, including TREM1 signaling, as top canonical pathways. In addition, a set of genes, including BRD4, was identified as the predicted transcriptional upstream regulators of these canonical pathways. BRD4, an epigenetic reader that recognizes acetylated lysine residues, plays a critical role in the transcriptional regulation of inflammatory genes and has been previously reported to regulate TREM1 expression. Based on this, we investigated whether global ischemia activates BRD4 and neuroinflammation through epigenetic regulation of TREM1. We first validated that the protein level of BRD4 correlates with changes in TREM1 mRNA and protein expression in the hippocampal CA1 using RT-qPCR and Western blot analysis. BRD4 was significantly increased at 48 h after global ischemia, coinciding with elevated TREM1 mRNA and protein levels. Consistent with this, levels of pro-inflammatory cytokines were also significantly increased in the hippocampal CA1 at the same time point after global ischemia, implicating enhanced TREM1-mediated neuroinflammation. Using immunohistochemistry, we found that BRD4 co-localized with TREM1-positive cells, and ChIP assays revealed that BRD4 binds to the TREM1 promoter following global ischemia. Importantly, BRD4 inhibition by JQ1 reduced TREM1 expression and pro-inflammatory cytokine levels, attenuated astrocyte and microglia activation, and ultimately protected neurons in the hippocampal CA1 after global ischemia. Taken together, these findings suggest that BRD4 upregulates TREM1 expression, which in turn enhances neuroinflammation and promotes neuronal death in the hippocampal CA1 after global ischemia. This implicates BRD4-dependent epigenetic regulation of TREM1 as a potential therapeutic target for this devastating neurological disease.

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Poster

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Title: Electrical Stimulation Enhances Angiogenesis and Vascular Integrity in Endothelial Cells

Authors: *B. KIM[1], J. PARK[2], H. KIM[3], R. AFZAL[4], J. CHOI[5];
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Abstract: Ischemic Stroke is a leading cause of death and disability worldwide, with ischemic stroke accounting for over 85% of cases. It occurs due to an obstruction in cerebral blood flow, leading to oxygen and nutrient deprivation, neuronal death, and irreversible damage. Angiogenesis, the formation of new blood vessels, is a key recovery mechanism that improves oxygen delivery, reduces infarct volume, and restores neurovascular function. Electrical stimulation(ES) has emerged as a potential strategy for enhancing angiogenesis, but its application in ischemic stroke treatment remains underexplored. In this study, we investigated the effects of different electrical stimulation(ES) conditions on angiogenic responses using human umbilical vein endothelial cells (HUVECs) and the ELEXIR stimulation device (Nueyne Co., Ltd.), which has received FDA approval for migraine treatment. Cells were stimulated at a constant voltage of 8 V under three distinct frequency-intensity combinations: 2 Hz at 5 mA, 20 Hz at 7 mA, and 40 Hz at 9 mA, all delivered with a pulse width of 250 microseconds. To evaluate angiogenic behavior, we conducted a wound healing assay, where ES was applied for 30 minutes following cell seeding and migration was measured after 10 hours. A tube formation assay was also performed, with stimulation administered 3 hours post-seeding and tube morphology assessed at 1-hour intervals. In addition, we analyzed the expression of blood-brain barrier (BBB)-associated proteins, ZO-1and VE-cadherin, via immunocytochemistry to assess vascular stability. The results revealed no significant differences in cell migration or BBB protein expression among the ES and control groups. However, 20 Hz stimulation significantly enhanced tube formation, suggesting increased vascular network development under this condition. In contrast, 40 Hz stimulation resulted in a noticeable reduction of ZO-1 and VE-cadherin expression, indicating a potential destabilizing effect on endothelial barrier function at higher frequencies. In this study, we demonstrate that electrical stimulation enhances angiogenesis under specific stimulation conditions. Notably, stimulation at 20 Hz was most effective in promoting vascular network formation without compromising endothelial integrity. These results support the potential of optimized electrical stimulation(ES) as a novel therapeutic strategy to enhance vascular regeneration in the context of ischemic stroke recovery.

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Poster

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Title: 4d nucleome remodeling orchestrates transcriptional reprogramming after ischemic stroke

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Abstract: The pathophysiology of ischemic stroke unfolds across temporally distinct phases, each characterized by unique transcriptional programs. Whether these transitions are scaffolded by coordinated changes in higher-order genome architecture remains poorly understood. Using peri-infarct cortex from adult male mice subjected to transient focal ischemia and reperfusion for 6h or 24h, we performed high-resolution Hi-C, which captures genome-wide chromosomal confirmation status. Sham operated mice served as a control. The data was analyzed by integrating chromatin contact maps (Juicer, eigenvectors, Arrowhead TADs and HiCCUPS loops) with gene expression (coding and non-coding) and regulatory annotations (enhancers, super-enhancers, promoters, CTCF). The data showed that stroke leads to multiscale 4D nucleome remodeling showing altered spatial genome organization over time indicating transcriptional reprogramming. At the compartment level, there was a high shift from A to B at 6h, partially reverting to A by 24h. At 10kb resolution, many TADs exhibited substantial gain or loss. Gained TADs were significantly shorter, yet denser in genes and enhancers across both time points, enriched for neurodevelopmental and synaptic pathways such as Wnt signaling, synapse assembly and glial proliferation. Lost TADs were larger and enriched for inflammation and apoptosis such as p53 signaling and T cell response, especially at 24h. Chromatin loop dynamics showed distinct temporal and length-dependent patterns. While loop gains were detected at both 6h and 24h compared to Sham, the 6h timepoint showed a stronger gain response. In contrast, 24h exhibited a more loop loss compared to Sham than what was observed at 6h. At 6h, short loops had stronger association with enhancers and promoters and by 24h, longer loops gained were enriched for super-enhancers. Loop-associated genes showed enrichment in stroke-related pathways including stem cell differentiation, CNS development, neuron differentiation and migration. Notably, non-coding RNAs were prominently upregulated across all categories of TADs and loops. Overall, stroke induced a coordinated, multilayered remodeling of the 4D nucleome that aligns with dynamic waves of gene activation, inflammation, and repair and non-coding RNAs as key controllers of the post-stroke regulatory landscape.

Disclosures: H. Namous: None. **R. Vemuganti:** None.

Poster

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Title: Genome-wide mapping and functional characterization of R-loops after ischemic stroke

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Abstract: Ischemic stroke induces rapid and extensive changes in transcriptional regulatory mechanisms across diverse genomic regions of the brain. These alterations fine-tune gene expression patterns to orchestrate key post-stroke pathological responses. Precise control of these processes is critical for limiting brain damage and facilitating long-term neurological recovery. During transcription, RNA can hybridize with the DNA template to form three-stranded RNA-DNA hybrids, termed R-loops. Accumulation of R-loops leads to altered transcriptional fidelity, genomic instability, and DNA damage. We currently explored the effect of stroke on R-loop formation in adult mice subjected to transient middle cerebral artery occlusion. Genome-wide R-loop accumulation was significantly increased in the peri-infarct cortex of mice subjected to focal ischemia compared to sham. Additionally, DNA-RNA immunoprecipitation followed by cDNA conversion coupled to high-throughput sequencing (DRIPc-seq) revealed differential accumulation of R-loop peaks across promoter, gene body, terminator, or intergenic regions of the genome. Further, gene ontology analysis of genes associated with significantly altered R-loop peaks in stroke cohort indicated that they regulate several biological pathways, including metabolism, intracellular signal transduction, dendrite development, morphogenesis, and organization, which play a significant role in neurologic and functional recovery after stroke. Overall, our results indicate a potential role of R-loops in modulating ischemic stroke pathophysiology.

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Poster

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Title: Ras-mapk pathway signaling supports post-ischemic neural regeneration and plasticity

Authors: *N. AICH, M. BRADFORD, B. AKHTER, R. MARQUEZ-ORTIZ, E. WILLIAMS, K. BROWN, K. RODGERS;
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Abstract: Background: Stroke causes impairment of local brain activity and contributes to long-term disability in millions of stroke survivors across all age groups. However, it has been suggested that the young brain has a greater ability for repair compared to adults, which our previous studies have supported. Although ischemic stroke causes neuronal loss, there is increased plasticity in the juvenile brain contributing to post-ischemic functional recovery, which we do not observe in adult mice. According to our preliminary studies, one mechanism may involve brain derived neurotrophic factor (BDNF)-TrkB (tropomyosin related kinase B) activation of RAS-MAPK pathway signaling, which promotes neuronal proliferation, survival, and differentiation in the juvenile brain. We observe opposing, age-related differences in RAS-MAPK pathway activation in adults, with pathological and damaging effects.
Methods: To explore age-related differences in RAS-MAPK signaling, we used the middle cerebral artery occlusion (MCAO) model in juvenile and adult mice, and performed immunohistochemistry, gene expression profiling, western blot, and *in vivo* electrophysiology.
Results: We found increased numbers of neurons and neuronal BDNF and pTrkB expression at 72hr and 30d in the stroke-injured striatum of juveniles, which was coupled with enhanced electrophysiological recovery at 30d. In contrast, we found astrocyte proliferation and increased astrocytic BDNF-pTrkB expression along with impaired electrophysiological recovery at all time points in adults. Further, we found upregulated BDNF gene expression in MCAO-injured juveniles and downregulation in adults at 14d following stroke, during the time before recovery of neuroplasticity was observed at 30d. Finally, we detected elevated RAS-MAPK protein expression in both groups. However, stroke-induced RAS-MAPK pathway activation was likely driven by astrocytes in adults, compared to neurons in juveniles.
Conclusions: These results suggest BDNF-TrkB mediated RAS-MAPK signaling is activated in an age-dependent manner, promoting healthy neuronal regeneration and functional recovery in juveniles and dysregulated/toxic activation that is damaging and pathological in adults.

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Poster

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Title: The TLR2-NF-κB-cIAP2 Pathway Promotes Oligodendrocyte Survival in Ischemic White Matter Injury

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Abstract: Subcortical Ischemic White Matter Injury (SIWMI) and associated oligodendrocyte (OL) loss is a significant and highly prevalent, yet often overlooked, cause of old-age cognitive decline. Through prior studies, we have demonstrated the protective role of Toll-like Receptor 2 (TLR2) in SIWMI, reducing lesion size and promoting oligodendrocyte progenitor cell (OPC) migration into the lesion through interaction with high-mobility group box 1 (HMGB1), which is released by injured OLs. We aimed to dissect the precise intracellular signaling pathway through which TLR2 exerts its oligodendrocyte-protective function. We applied oxygen glucose deprivation (OGD) to primary OL cultures and observed the phosphorylation of downstream signals of TLR2, such as ERK1/2, p38, NF-κB, CREB, and AKT, in response to the administration of Pam3, a TLR2 agonist. Upon treatment with Pam3, the phosphorylation and activation of ERK1/2, p38, NF-κB and CREB, but not AKT, was observed. Subsequently, by utilizing chemical inhibitors and siRNA knockdown for the four signals activated upon treatment with Pam3, we discovered that p38 and NF-κB, and not ERK1/2 or CREB, were essential for the OL survival-promoting effect induced by TLR2 stimulation. We focused on the anti-apoptotic proteins cellular inhibitor of apoptosis protein 2 (cIAP2) and bcl2, each downstream of NF-κB and p38. Upon overexpressing p38 or NF-κB in cultured OLs, we found that cIAP2 but not bcl2 increased in expression. NF-κB activation was sufficient to increase cIAP2 levels and promote OL survival in OGD regardless of p38 knockdown, but not vice versa, indicating that the protective effect of p38 was dependent of NF-κB. cIAP2 overexpression exerted OL-protective effects even when NF-κB was inactivated, indicating that cIAP2 is a necessary downstream factor for TLR2- NF-κB to mediate OL survival. The in vitro findings were further validated through bulk RNA-seq comparisons of OGD vs OGD+Pam3 OLs. Differentially expressed gene (DEG) and pathway enrichment analysis showed that the NF-κB pathway and related genes, including cIAP2, were significantly upregulated in Pam3-treated OLs. To confirm the OL-survival promotion by NF-κB and cIAP2 in vivo, we administered AAV-mMBP-Flag-IKK2 into the right internal capsule of C57BL/6 mice, three weeks after ischemic injury induced by stereotaxic injection of L-NIO. NF-κB activation was sufficient to reduce lesion size and rescue the decline in neurological function caused by L-NIO. Collectively, the findings demonstrate that TLR2, through downstream signals NF-κB and cIAP2, promotes the resistance of OLs to ischemic injury, suggesting its value as a therapeutic target in SIWMI.

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Poster

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Support: VA OSR 1IK2BX005369

Title: Unraveling Arctic Ground Squirrel Metabolic Resilience: Transcriptomic and Mitochondrial Insights into ATP5G1-Mediated Neuroprotection in Stroke

Authors: *L. GUPTA[1], M. ZHANG[2], S. WON[3], K. ARKELIUS[2], N. SINGHAL[4];
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Abstract: Arctic ground squirrels (AGS) are extreme hibernators capable of withstanding months of freezing temperatures by suppressing metabolic rate. Hibernation is characterized by hypoxia and low cerebral blood flow and interrupted by bouts of arousal in which perfusion is quickly restored, yet AGS suffer no ischemic or reperfusion injuries. Laboratory models of stroke or anoxic brain injury demonstrate decreased neuronal injury in AGS. In vitro neurons derived from Arctic ground squirrel stem cells also possess remarkable tolerance to metabolic stressors mediated in part by mitochondrial adaptations. We discovered ATP5G1 to be an important cytoprotective factor following metabolic stressors in vitro. We sought to understand if ATP5G1 is also neuroprotective in preclinical mouse stroke models, mitochondrial mechanisms of ATP5G1 cytoprotection, and to identify additional factors critical for AGS metabolic resilience. We used RNA sequencing to characterize the transcriptomic landscape of mouse and Arctic ground squirrel neural progenitor cells following exposure to the complex I inhibitor, rotenone, and thapsigargin, an ER stress inducer. Blue native gel electrophoresis was used to characterize mitochondrial subunit expression following stressor exposure. The transient middle cerebral artery occlusion model was used to assess the neuroprotective effects of AAV-mediated ATP5G1 overexpression. Rotenone and thapsigargin induced numerous overlapping genes in mouse and AGS neural progenitor cells including genes canonically involved in mitochondrial metabolism and proteasomal responses. Unique genes upregulated in AGS neural progenitor cells included those involved protein lipidation and lipoprotein biosynthesis. In addition, mouse and AGS cells exhibited differential regulation of mitochondrial subunits in response to rotenone. Interestingly, overexpression of ATP5G1 in mice resulted in 40% reductions in infarct size at 24 hours following stroke. These findings reveal mechanisms of unique metabolic adaptations in Arctic ground squirrels. The significant reduction in infarct size observed with ATP5G1 overexpression in mice highlights its therapeutic potential for treating stroke and suggests novel strategies for metabolic resilience and neuroprotection in humans.

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Poster

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Title: Astrocytic Slc4a4 maintains neurovascular unit integrity and cognitive function in mouse model of vascular dementia

Authors: *J. CERDA III;
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Abstract: The neurovascular unit (NVU) is a cellular network that maintains brain homeostasis by forming the blood-brain-barrier (BBB) and regulating CNS function. Disruption of NVU integrity contributes to a range of acute and chronic cerebrovascular conditions such as stroke and vascular dementia (VaD). We previously demonstrated that astrocytic SLC4A4 (Solute Carrier Family 4 Member 4) is essential for maintaining BBB integrity following acute ischemic injury. However, whether Slc4a4 regulates NVU function in chronic cerebrovascular conditions like VaD remains unclear. To investigate the function of Slc4a4 in VaD pathophysiology, we utilized both loss-of-function (astrocyte-specific Slc4a4 conditional knockout, Slc4a4-cKO, mouse model) and gain-of-function (viral Slc4a4 expression) approaches in established chronic hypoperfusion mouse models of VaD. Following VaD injury, Slc4a4-cKO mice exhibit significant neuronal loss, increased microglial cell numbers with enhanced ramified morphology, reduced astrocyte activation, with severe cognitive deficits in learning and memory behavioral tasks. In contrast, astrocytic Slc4a4 overexpression following VaD injury markedly alleviates neuronal loss, reduces microglial activation, and improves cognitive performance. To elucidate the mechanism underlying the function of Slc4a4 in VaD, we performed bulk RNA sequencing of Slc4a4-cKO mice that underwent VaD injury. This analysis revealed enrichment of differentially expressed genes associated with cognitive deficits and neurotransmitter dysfunction, providing potential mechanistic insight into how Slc4a4 loss contributes to VaD pathology. Taken together, these studies suggest that Slc4a4 is an essential regulator of NVU integrity in VaD and reveals its therapeutic potential for targeting VaD-related cognitive dysfunction.

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Poster

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Title: Cxcr2-dependent neuroprotective and regenerative effects of glia-like human mesencymal stem cells in vascular dementia models

Authors: *M.-S. CHANG[1], M.-J. LEE[1], S.-H. NAM[1], E. LEE[2], Y. RYU[2], L. SEONJU[2], H. SEO[3], D. HAN[4], S.-H. KOH[5];
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Abstract: Vascular dementia, the second most common form of dementia, results from chronic cerebral hypoperfusion and is associated with progressive neuronal loss, particularly in the hippocampus, leading to cognitive impairment. Here, we investigated the therapeutic potential of glia-like human mesenchymal stem cells (ghMSCs), generated from human MSCs through small molecule-induced fate conversion without genetic modification. Transcriptomic and proteomic profiling revealed that ghMSCs acquired astrocyte-like and neuroprotective signatures. Using both an ex vivo organotypic hippocampal slice model of ischemia and an in vivo mouse model of bilateral common carotid artery stenosis (BCAS), we show that ghMSC transplantation significantly enhances neuronal survival and cognitive function compared to naïve hMSCs. Behavioral testing in BCAS mice demonstrated improved cognitive performance following ghMSC treatment. Importantly, administration of a CXCR2 antagonist abolished these neuroprotective and regenerative effects in both models, indicating that CXCR2 signaling is a key mediator of ghMSC function. These findings identify ghMSCs as a promising therapeutic strategy for vascular dementia and highlight CXCR2 signaling as a critical molecular pathway underlying their efficacy.

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Poster

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Topic: D.02. Ischemia

Support: R03NS128372

Title: Novel model to investigate neonatal hypoxic-ischemic brain injury demonstrates hippocampal and white matter inflammation

Authors: K. LANGER[1], E. TIEMEIER[1], E. HARMON[2], J. HENRY[2], A. FINEBERG[2], R. DIETZ[2], A. L. DINGMAN[2], *N. QUILLINAN[3];
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Abstract: Neonatal hypoxic ischemic (HI) brain injury is a leading cause of infant mortality and lifelong disability; however, the field is lacking a model that simulates global cerebral ischemia (GCI) and reperfusion injury, which is essential to the pathophysiology of HI. Here we developed and characterized a novel rodent model of cardiac arrest and cardiopulmonary resuscitation (CA/CPR) to induce GCI that produces glial inflammation, white matter alterations, and memory impairment. Rat pups underwent CA/CPR or sham procedure on postnatal day 10. For CA/CPR, rat pups were anesthetized and intubated. Asystole was induced with intravenous (IV) KCl and maintained for 12 minutes. Resuscitation involved 100% O_2, chest compressions, and IV epinephrine. Sham controls underwent anesthesia, intubation, and jugular access but without KCl, epinephrine, or compressions. To assess cell death, we conducted Fluorojade C (FJC) staining of the CA1 region of the hippocampus (Hp) 7 days post-CA/CPR (POD7). Also, at POD7, we conducted immunohistochemistry and confocal microscopy to assess microglia and astrocyte gliosis in the CA1 and corpus callosum (CC). Gliosis was quantified with percent positive pixel analysis on ImageJ. To assess myelin properties at POD7, we conducted spectral microscopy in the CC and calculated a Polarity Index (PI) which is inversely proportional to cholesterol content, a key component of normal myelin. At POD14, we conducted a behavioral assessment - the Contextual Fear Conditioning (CFC) task - which is an indicator of Hp-dependent memory. In the CA1, there is no cell death after CA/CPR at POD7, indicated by no significant difference in FJC staining ($p=0.316$), but there is evidence of microglia and astrocyte gliosis ($p=0.018$; $p=0.045$, respectively). We found deficits in Hp-dependent learning by POD14 as indicated by reduced freezing during the CFC ($p= 0.048$). In the CC, confocal and spectral microscopy showed that by POD7, astrocyte gliosis and PI were significantly higher in the lateral CC after CA/CPR compared to sham ($p=0.020$ and $p=0.036$). In our novel model of neonatal GCI using CA/CPR, we see resilience to cell death in the CA1 region of the Hp 7 days after injury; however, there is evidence of prolonged functional deficits in Hp-dependent memory. Given our findings of microglia and astrocyte gliosis in the CA1 region as well as astrogliosis and WM microstructure changes in the lateral CC, it's possible that these deficits may be driven by inflammation rather than gross cell death. Future work is necessary to better understand the functional consequences of neonatal HI as well as the contribution of glial inflammation to the pathophysiology of this injury.

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Poster

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Topic: D.02. Ischemia

Title: From first-responders to foam cells: profiling microglial responses to ischemic stroke using high-plex spatial proteomics

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Abstract: As the brain's resident immune cells, microglia play a central role in the neuroinflammatory response to ischemic stroke, undergoing dynamic morphological changes and shifts in protein expression depending on both post-stroke timepoint and proximity to the infarct. To capture these evolving responses, we used the high-plex CosMx™ Mouse Neuroscience Protein Panel in a mouse model of ischemic stroke to spatially profile microglial behavior across three post-ischemic timepoints. Transient middle cerebral artery occlusion (tMCAO) was induced in adult male mice using the filament insertion method, followed by reperfusion. Stroke severity was confirmed using a Bruker Biospec 70/20 7.0T MRI system. Brain tissues were harvested at 24 hours, 2 weeks, and 4 weeks post-stroke (n=3 per timepoint), fixed, and then cryosectioned at 10 µm for spatial proteomic analysis using the CosMx Spatial Molecular Imager (SMI). The extensive protein panel, which includes several microglial markers, was critical for capturing the complexity of these cells' morphology and function. We focused on seven key proteins (IBA1, CD11b, CD45, DAP12, P2RY12, CD68, and TMEM119) to visualize microglial processes more effectively. This approach enabled more accurate cell segmentation and helped distinguish protein expression associated with cellular processes from that of the soma, and how these profiles might change over time. We then performed a differential protein expression analysis using InSituDiff to identify proteins whose expression was perturbed across brain regions and timepoints. In particular, we aimed to investigate and characterize microglial states near the developing glial scar. These regions contained dense accumulations of MBP inside of and adjacent to APOE+/Cathepsin B+ cells that emerged within the scar most prominently at the 4 week timepoint, which suggest the presence of foam cell-like responses. By integrating high-plex spatial proteomics, multi-marker-informed cell segmentation, and spatially resolved differential expression analysis, our study offers a nuanced view of microglial behavior throughout stroke recovery. These findings underscore the value of spatial biology tools in uncovering cellular interactions and responses that may be overlooked in bulk or dissociated tissue analyses, providing a foundation for future investigations into post-stroke neuroinflammation.

Disclosures: K.F. Young: A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **H.W. Morrison:** A. Employment/Salary (full or part-time): University of Arizona, College of Nursing, Tucson, AZ. **K.P. Doyle:** A. Employment/Salary (full or part-time): University of Arizona, College of Medicine, Tucson, AZ. **L. Wu:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **A. Wardhani:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **A. Heck:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **C. Williams:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **M. Patrick:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **M. Hoang:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA. **J. Beechem:** A. Employment/Salary (full or part-time): Bruker Spatial Biology, Seattle, WA, USA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology, Seattle, WA, USA.

Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

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Topic: D.02. Ischemia

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Title: Mitochondrial Quality Control Mechanisms drive Microglial Polarization following Oxygen-Glucose Deprivation And BzATP Exposure.

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Abstract: Ischemic injury is a leading cause of death and long-term disability worldwide, largely due to mitochondrial dysfunction that drives neuronal death and neuroinflammation. Microglia, the brain's resident immune cells, detect damage-associated molecular patterns (DAMPs) and initiate inflammatory responses in a process termed polarization. Accumulation of fragmented mitochondria is associated with chronic neuroinflammation and the development of neurodegenerative diseases post-stroke. Mitochondrial quality control mechanisms, such as the PINK1/Parkin mitophagy pathway, degrade dysfunctional mitochondria and are understood to protect against deleterious inflammation. However, Parkin may also initiate inflammation by facilitating IKK-complex formation on mitochondria, a key step in NF-κB activation and microglial polarization. To investigate the contribution of mitochondrial quality control mechanisms on polarization, primary WT and Parkin-/- microglial cultures were exposed to BzATP, a known post-ischemia DAMP, and oxygen-glucose deprivation and reperfusion (OGD/R). Live-cell imaging revealed mitochondrial depolarization and fragmentation post-BzATP exposure. Exposure to BzATP also increased p62 and LC3B/A protein expression levels, suggesting induction of mitophagy but not its resolution, in both genotypes. Conversely, OGD/R and BzATP increased expression of inflammatory markers Arginase1, and NLRP3 in WT but not Parkin-/- microglia. WT microglia mitochondrial morphology fragmented, then recovered following OGD/R, whereas Parkin-/- microglia contained fragmented mitochondria morphologies which were static across control and stress conditions. Additionally, the Seahorse Mito Stress Test revealed that BzATP increased maximal respiration in the WT, but not the Parkin-/-. Ongoing experiments are investigating the role of the mitochondrial calcium uniporter on mitochondrial membrane depolarization and polarization via matrix calcium influx following BzATP exposure.

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Poster

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Topic: D.02. Ischemia

Title: Time dependent microglial phenotypes influence neuroplasticity associated gene expression in post stroke neurons

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Abstract: Stroke is a leading cause of long-term disability, creating the need for effective therapies to facilitate recovery. Microglia, the resident immune cells of the central nervous system, are among the first responders to ischemic damage and play pivotal roles in tissue repair. Upon ischemic injury, activated microglia subtypes influence critical post-stroke processes including cytokine and chemokine production, neurotrophic support, synapse elimination, and

neuro-immune modulation in a spatiotemporal manner. With microglial phenotypes being spatiotemporally dependent, understanding what molecular pathways facilitate microglial roles in these post-stroke processes could identify potential therapeutic targets. What cell-cell communication is occurring between microglia and neurons? To examine this question, we used 6 publicly available single cell RNA-sequencing datasets from stroke studies were processed to perform additional analysis. Despite vastly different preparations, datasets successfully integrated, forming unique clusters. Microglia were further subclustered and suggest temporally specific phenotypes. Further annotation of microglial phenotypes will allow for the analysis of neuronal interactions via cell-cell communication analysis. We anticipate our integrated single-cell study will reveal how microglia orchestrate temporally precise pro-regenerative programs in neurons via defined ligand-receptor channels and/or transcription factor hubs. Targeting pathways, or upstream microglial regulators will enhance our knowledge on how microglia contribute to post-stroke repair.

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Poster

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Topic: D.02. Ischemia

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Title: Investigating dynamic changes to cell viability and morphology after ischemia-reperfusion injury in 3D cortical microtissues

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Abstract: Reperfusion after stroke is essential and necessary to prevent further infarction. However, 55% of patients suffer from poor outcomes after reperfusion, and underlying mechanisms responsible for differences in tissue survival are not well understood. Here, a self-assembled, high-throughput 3D in vitro rat cortical microtissue model was used to determine temporal effects of ischemia-reperfusion injury on viability and morphology. On culture day 13, microtissues were exposed to oxygen-glucose deprivation (OGD) for 24 hours, followed by 1 day, 3 days, and 6 days of reperfusion (OGD/R). Viability assays were performed in OGD/R microtissues and compared to age-matched controls. ATP content was significantly decreased in OGD/R microtissues at each timepoint. Caspase 3/7 activity was significantly increased in OGD/R 1-day microtissues, then it returned to baseline levels at later timepoints. Lactate dehydrogenase content was significantly increased in OGD microtissues, peaked at 1 day of

reperfusion, followed by a decrease at 3 days and back to baseline levels after 6 days of reperfusion. A significant decrease in glutathione levels in OGD/R microtissues at each timepoint indicated an increase in reactive oxygen species production after injury. Furthermore, we observed a distinct morphological response following reperfusion. Directly after OGD, microtissues displayed neurite network injury through the breakdown of Beta-3-tubulin cytoskeletal structures and hyperreactive astrocytes with enhanced glial fibrillary acidic protein expression, thicker branches, and enlarged somas. After 1 day of reperfusion there was a split in microtissue phenotypes. A vulnerable population showed signs of persistent injury with phenotypic characteristics similar to OGD. A resilient population expressed phenotypes similar to pre-injury with strand-like cytoskeletons and homeostatic astrocytes. The expression of phenotypes was paired between neurons and astrocytes across the microtissue populations, and the phenotypes were sustained longitudinally for 6 days. In this study, we have shown that ischemia-reperfusion injury induced distinct temporal changes to metabolism, apoptosis, and oxidative stress. The 60% vulnerable and 40% resilient microtissue split characterized by neuron and astrocyte morphologies resembled patient outcomes after stroke. Future characterization of biological mechanisms contributing to OGD/R recovery may lead to the development of novel therapeutics that mitigate reperfusion injury following stroke.

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Poster

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Program #/Poster #: PSTR386.17/I12

Topic: D.02. Ischemia

Support: Roneet Carmell Memorial

Title: Ultra-short caloric restriction modulates neuroprotective metabolites during cardiac arrest and resuscitation

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Abstract: Intermittent fasting can protect against cardiovascular disease, however, benefits of ultra-short caloric restriction (CR) remain underexplored. We previously showed that ultra-short CR improves survival and neurological recovery after cardiac arrest (CA) and resuscitation in a rat model. We hypothesize that ultra-short CR leads to shifts in metabolism that yield neuroprotection and cardioprotection, which may be revealed by the analysis of serum metabolites. To test this, we used a rodent model of CA and resuscitation, with a focus on

untargeted metabolomics. We isolated serum before and after CA from different regions of the body to reveal organ-specific metabolite profiles. Male Wistar rats underwent a 75% CR 16 hours pre-CA (n=9), while non-CR rats received ad libitum food (n=7). Thirty minutes before CA, blood samples were drawn from the femoral artery and jugular vein. Rats underwent 8 minutes of asphyxial CA, followed by cardiopulmonary resuscitation. Post-CA blood samples were collected ~12 minutes after resuscitation. Metabolomic profiling was conducted using a Xevo G-XS QTOF mass spectrometer to quantify features and SIRIUS software for identification. CR rats exhibited significantly higher levels of acetylcarnitine (AC) compared to non-CR rats at the pre-CA timepoint and at both collection locations ($p<0.02$). P-coumaric acid (pCA) levels were higher after CR compared to non-CR rats at both time points and locations ($p<0.02$), with additional increase post-CA compared to pre-CA at both locations ($p<0.03$). Indoleacrylic acid (IA) was decreased following CA only in non-CR rats at both locations, while levels were similar between pre-CA and post-CA blood in the CR rats ($p<0.03$). CR demonstrates higher levels of L-Tyr, IA, and Methyl Indole-3-Acetate in arterial blood compared to venous blood ($p>0.05$), whereas no significant differences between venous and arterial blood were observed in non-CR rats. Our results demonstrate that ultra-short CR induced a distinct metabolic profile compared to non-CR rats. A rise in AC, pCA, and stabilization of IA observed in CR rats suggests stronger antioxidant capacity and reduced inflammation as a result of ultra-short CR. CR-induced modulation of metabolites between arterial and venous blood indicates increased tissue-level consumption of protective metabolites, including by the brain. Ultra-short CR leads to potent effects on serum metabolites in different regions of the body, possibly serving as one of the mechanisms behind the neuroprotective effects seen after ultra-short CR. Future studies can explore enrichment of these crucial metabolic pathways to improve outcomes after global ischemic injury.

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Poster

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Topic: D.02. Ischemia

Support: Roneet Carmell Memorial Endowment Fund

Title: Multimodal analysis of neurovascular coupling may be predictive of post-ischemic brain injury

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Abstract: Ischemic brain injury as a result of cardiac arrest (CA) impairs neurovascular coupling (NVC), disrupting the brain's ability to meet metabolic demand with adequate cerebral perfusion. This can result in worse outcomes. NVC can be measured by simultaneously recording brain electrophysiology and cerebral hemodynamics. Prior studies have examined NVC using either intracranial pressure (ICP) or cerebral blood flow (CBF) waveforms in subjects under burst-suppression on electroencephalography (EEG). Although EEG bursts have been independently linked to either ICP or CBF oscillations, minimal studies have measured NVC through a consolidated display of EEG bursts with ICP, CBF, and the concomitant changes in brain metabolism. This study aims to measure NVC through the dynamics of cerebral metabolic rate of oxygen ($CMRO_2$), CBF, and ICP immediately after acute ischemic brain injury to prognosticate post-CA neurological outcome. Male rats were anesthetized, intubated, and mechanically ventilated. Neural activity was recorded using electrocorticography (ECoG). Polyethylene tubing was placed into a burr hole for ICP monitoring. Laser speckle imaging and spatial frequency domain imaging were used to measure CBF and cerebral oxygenation, respectively, which were used to calculate $CMRO_2$. After 7 minutes of asphyxial CA, cardiopulmonary resuscitation was performed. Burst-suppression ratio (BSR), a quantitative measure of post-CA ECoG recovery, was measured at 18-25 minutes post-resuscitation as an early ECoG-based marker of neurological recovery. Results show that amplitudes of the $CMRO_2$, CBF, and ICP oscillations to ECoG bursting are significantly attenuated post-CA, indicating impairment in cerebral metabolism and NVC immediately after ischemic brain injury ($CMRO_2$; n=6, p=0.020. CBF; n=7, p=0.001. ICP; n=7, p=0.001). Also, during pre-CA, relative CBF changes in response to ECoG bursting are directly correlated to BSR area under the curve (AUC; n=6, R=0.937, p=0.006) and slope (n=6, R=0.919, p=0.009). Post-CA, the recovery of this CBF response to ECoG bursting is also directly correlated to BSR AUC (n=6, R=0.898, p=0.015) and slope (n=6, R=0.862, p=0.027). This suggests that more efficient pre-CA NVC and an improved post-CA NVC recovery may be associated with faster ECoG recovery, which may be associated with improved neurological outcome. In conclusion, our results highlight the relationship between neural activity, $CMRO_2$, CBF, and ICP in a normal and post-ischemic state, demonstrating the potential utility of monitoring such dynamics to improve understanding, assessment, prognostication, and guide treatment during acute brain injury.

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Poster

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Topic: D.02. Ischemia

Support: NIH Grant R01MH086638

Title: Simulations of spreading depression in neocortical microcircuits using human capillary data

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Abstract: Spreading depolarization (SD) is characterized by a wave of neuronal depolarization that propagates through grey matter at ~2-7mm/min. SD is involved in many neurological conditions, including; ischemic stroke, complex migraine and traumatic brain injury. Here we develop a network model that includes concentrations of relevant ions to simulate and compare SD under these different pathologies.

We used NEURON RxD to simulate Hodgkin-Huxley style neuron model augmented with homeostatic mechanisms, including Na^+/K^+-ATPase, NKCC1, KCC2, and dynamic volume changes. We modeled Na^+, K^+, Cl^-, and O_2 concentrations, with blood vessels locations providing point sources of O_2 and O_2-dependent clearance of K^+. We created a network model with NetPyNE, using 13,000 point neurons representing ~1mm^3 cortex (layers 2-6). Connectivity was based on prior cortical modeling, with connection weights scaled to account for differences between conductance based and current based neuron models. We simulated SD in three conditions: (1) extracellular K^+ concentrations, similar to experimental application of KCl to trigger SD and thought to model migraine aura. (2) hypoxia, similar to experimental oxygen glucose deprivation used to model the core of an ischemic stroke. (3) elevated K^+ with mild hypoxia, to model the penumbra of an ischemic stroke during SD.

A 2.0 x 2.3 cm cross-section human V1 cortex immunostained for CD34 (an endothelial cell marker) was used to determine oxygen sources for the model. In total 918 capillaries were histologically identified, with average density of 199.6/cm^2, and capillary cross-sectional area of 16.7±11.9μm^2. Elevating K^+ could induce SD in all cortical layers, although the speed of propagation differed between layers. Hypoxic conditions with elevated K^+ spread faster than in normoxic conditions and spread both through extracellular K^+ diffusion and through network connectivity. Our model also showed that capillaries affected neurons' ability to maintain homeostasis and physiological firing.

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Poster

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Topic: D.02. Ischemia

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Title: Effect of entinostat against the inflammation induced by the permanent ischemic stroke in diabetic rats

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Abstract: Stroke is a leading cause of death and disability worldwide; it is classified into ischemic and hemorrhagic, with ischemic being the most prevalent. The therapeutic options are very limited, and a reduced number of patients have access to these treatments, resulting in a high incidence of permanent ischemic stroke. There are some risk factors contributing to ischemic burden. Diabetes mellitus increases 2 to 6 times the probability of suffering a stroke, and the severity and mortality of ischemic stroke. Inflammation is an essential contributor to brain damage in ischemic stroke, and it is exacerbated by diabetes. Modulation of inflammation by inhibiting histone deacetylases (HDAC) results in a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines. In transient ischemic stroke, HDAC inhibitors have shown brain protection; however, their use in permanent ischemia plus diabetes has not been evaluated. In this work, we evaluated the effect of Entinostat (ENT), a class I HDAC inhibitor, on neurological deficit, HDAC and histone acetylase (HAT) activity, and inflammation markers in diabetic rats subjected to permanent ischemia. Different doses of ENT were administered to male Wistar rats (3 months) to evaluate its safety and efficacy in HDAC inhibition. Additionally, another group of rats was fed with a high-fat and glucose diet for 14 days, followed by a single dose of streptozotocin (STZ, 40 mg/kg, ip); seven days later, rats were subjected to 7 days of permanent ischemia. The safe and effective ENT dosage regimen was administered one hour after the onset of ischemia. The neurological deficit was evaluated seven days after ischemia, followed by the striatum and cortex obtention. HDAC and HAT activity were evaluated in nuclear fractions, whereas inflammation markers (TFN-α, p65, IL-1β, and IL-6) were evaluated in total homogenate by western blot. The 2.5 mg/kg dose of ENT every 24 h for 7 days shows inhibition of HDAC activity in the striatum without hematological toxicity. Moreover, this dosage regimen reduces the neurological deficit in diabetic animals subjected to permanent ischemia compared with non-treated animals; however, it did not show changes in HDAC activity compared to non-treated animals in the striatum and cortex after ischemia, whereas the HAT activity increased with the ENT treatment in both tissues. Moreover, ENT treatment decreases the expression of inflammatory markers in the cortex and reduces blood glucose levels. In conclusion, these results suggest that ENT treatment modulates the

inflammation, resulting in a reduction in neurological impairment in diabetic rats subjected to permanent ischemia.

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Poster

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RS-2023-00223591
RS-2023-00207946
RS-2024-00438628

Title: Decoding hippocampal subfield and glial responses in ischemia using single-cell transcriptomics

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Abstract: Stroke affects more than 12 million individuals worldwide annually, leading to lasting physical and cognitive impairments. The peri-infarct environment in the central nervous system, comprising glial and blood vessel cells, contributes to stroke progression. The hippocampal CA1 region is particularly vulnerable to ischemia, whereas the adjacent CA3-DG region exhibits different responses. Understanding the cellular and molecular alterations in these regions before and after ischemic insult can provide insights into stroke pathology and recovery mechanisms. We conducted single-cell RNA sequencing on Sprague-Dawley rats subjected to four-vessel occlusion (4-VO) surgery, a model of transient global cerebral ischemia, and compared them with normal control rats. Cellular composition and molecular signatures of the hippocampal CA1 and CA3-DG regions were analyzed under both ischemic and sham conditions to determine differences in glial and vascular cell responses. Following stroke, there was an elevation in pro-inflammatory microglial subtypes, with distinct differences in microglial pathways depending on the hippocampal region. A unique oligodendrocyte subtype emerged in the post-ischemic

hippocampus that was not present under normal conditions. Astrocytes maintained clear cluster characteristics under both normal and ischemic conditions without significant differences. Additionally, the proportion of cd74-positive pericytes increased specifically in the CA3-DG subfield after the 4-VO procedure. These findings highlight the diverse molecular signatures and functional differences of cells in specific hippocampal regions during global cerebral ischemia. Differences in cellular functions and composition between CA1 and CA3-DG subfields suggest that glial heterogeneity may contribute to regional differences in ischemic vulnerability. This study provides new insights into the role of glial and vascular cell populations in stroke pathology, which may inform future therapeutic strategies.

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Poster

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Title: Bio-orthogonal amino acid tagging reveals astrocyte-specific nascent proteomic changes in vivo post-transient global ischemia

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Abstract: Astrocytes are the most abundant glial cells that regulate the blood-brain barrier and provide trophic support to neurons. Ischemic brain injury leads to early and delayed consequences, including progressive neurodegeneration, which may involve astrocytes. However, a comprehensive understanding of the proteomic changes that occur in astrocytes following ischemic injury, while retaining their native state *in vivo*, is lacking. In this study, we selectively labeled newly synthesized (nascent) proteins in astrocytes, using cell type-specific in vivo bio-orthogonal non-canonical amino acid tagging (BONCAT). BONCAT utilizes Cre-mediated recombination to express a mutant methionyl-tRNA synthetase (MetRS), which incorporates the methionine analog azidonorleucine (ANL) into nascent proteins, enabling their purification using Click Chemistry. Diffuse cortical ischemia was induced in Aldh1l1-Cre-ERT2/MetRS-floxed mice by 20-minute bilateral common carotid artery occlusion (BCCAO), followed by a 3-week supplementation of ANL in drinking water. Azide-tagged proteins from the cortex were then Click-labeled with biotin-alkyne, enriched, and subjected to label-free quantitative mass spectrometry (LFQ-MS) for analysis. Specificity for nascent proteome in astrocytes was confirmed by fluorescent non-canonical amino acid tagging, while Western blot

analysis validated successful proteomic labeling compared to non-labeled controls. LFQ-MS of enriched azide-tagged proteins identified 772 nascent proteins specific to astrocytes, including established markers such as Aqp4, Aldh1l1 and Glul. LFQ-MS performed on mice that underwent BCCAO, compared to sham surgery, identified distinct proteomic changes occurring specifically in astrocytes. These included 84 upregulated (eg. Fus, Adrbk1) and 100 downregulated (eg. Ndufs8, Necap1) proteins, independent of sex and age. We also observed increased GFAP expression, suggesting sustained reactive gliosis and inflammation at 3 weeks following BCCAO. Importantly, astrocyte-specific proteomic changes in response to BCCAO, including increased astrocytic Fus, were not detectable at the level of whole brain proteomes. BCCAO increased myelination in the brain, evidenced by increased levels of myelin sheath proteins (Plp1, Mbp, Mag). Using ciBONCAT, we analyzed native state nascent proteins changes in astrocytes from adult mouse brains and identified BCCAO-induced proteomics alteration related to mitochondrial function, metabolism, and inflammation. These astrocyte-specific alterations, which persist weeks after ischemia, may highlight potential targets for therapeutic intervention.

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Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.23/J6

Topic: D.02. Ischemia

Title: Protective Role of Wnt3A in Hypoxia-Induced Blood Brain Barrier Injury

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Abstract: Hypoxia is associated with impairment of blood brain barrier (BBB) function in ischemia and traumatic brain injury. Hypoxia disrupt the BBB architecture leading to complex pathophysiological alteration in the brain. Brain microvascular endothelial cells (BMVEC), a major cell-type that shield the brain from toxins from blood, making a semipermeable barrier with tight junction proteins (TJPs) (occludin, claudin 5), adherent junctions and junctional adhesion molecules. The BMVEC forms a monolayer with other cells like astrocytes, microglia, *etc*., constituting the barrier for functional integrity. It is therefore critical to investigate the molecular mechanism of BBB impairment under hypoxia which is currently unknown. Recently, microRNA (miRNA) emerged as promising translatable therapy in many neurological diseases, however, it's role in hypoxia-induced BBB impairment remains obscure. We identified a panel of dysregulated miRNAs under 24h hypoxia compared to normoxia in human BMVEC using

hypoxia miRNA PCR array. We selected one upregulated miRNA, miR-195-5p for further investigation. Using bioinformatic tools, Tragetscan 8.0, we identified Wnt3A was a target for miR-195-5p which was significantly reduced under hypoxia. Wnt/β-catenin pathway contributed a pivotal role in BBB dynamics, however, Wnt3A's role in hypoxia-induced BBB disfunction remains unknown. Our hypothesis is that hypoxia-induced upregulation of miR-195-5p and down regulation of Wnt3A exacerbate BBB impairment and restoring the miR-195-5p-Wnt3A level mitigates the damage. In vitro experiments using human BMVEC exposed to 24h hypoxia caused BBB damage and dysfunction which include significant downregulation of Wnt3A, TJPs (claudin 5 and occludin), ve-Cadherin, β-catenin genes and proteins, increased BBB permeability and decreased electrical resistance in transendothelial electrical resistance (TEER) assay, compared to normoxia. Pretreatment with recombinant Wnt3A (rWnt3A) followed by 24h hypoxia reversed the process and restored BBB dysfunction. Furthermore, miR-195-5p mimic transfection exacerbated the BBB damage as stated above and miR-195-5p inhibition restored the damage indicated a potential role of Wnt3A-miR-195-5p in BBB coherence. Mechanistically, we found significant reduction of Stat3 phosphorylation under hypoxia and pretreatment with rWnt3A restored pStat3. Pharmacological inhibition of Wnt3A by salinomycin exacerbated the BBB dysfunction as stated above indicated a potential role of Wnt3A in BBB integrity. We concluded that miR-195-5p-Wnt3A-axis have therapeutic potential in hypoxia-induced BBB impairment in brain microvasculature.

Disclosures: S. Gupta: None. **W. Stewart:** None. **C. Hejl:** None. **R. Guleria:** None.

Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR386.24/J7

Topic: D.02. Ischemia

Support: NINDS, R01NS131484

Title: Toll-like receptor-mediated chemokine signaling promotes anti-inflammatory microglial activation and improved functional outcomes after pediatric stroke

Authors: *K. BROWN, B. AKHTER, M. BRADFORD, N. AICH, E. WILLIAMS, K. RODGERS;
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Abstract: Background: Stroke triggers activation of central and peripheral immunity through danger signaling from damaged and dying cells. These cells release endogenous molecules known as damage-associated molecular patterns, which are potent mediators of inflammation via toll-like receptor (TLR)-mediated activation of microglia. Once activated, microglia polarize towards proinflammatory and/or anti-inflammatory phenotypes, and this response has been shown to be both neurodegenerative and neuroprotective following brain injury. In adults, it has

been established that a proinflammatory microglial phenotype is more common during the acute response to stroke, which is detrimental to tissue regeneration and post-ischemic outcomes. However, we have found evidence after juvenile stroke, that early anti-inflammatory microglial activation is essential for long-term neuronal repopulation and functional recovery. While we find similar results to the literature in adults, with proinflammatory responses that result in tissue damage and functional impairments at acute and chronic time points. We aim to elucidate the critical neuroimmune signaling involved in these age-related differences, including microglial release of CXCL10, an interferon-γ-inducible chemokine that actively recruits microglia and CD4+/CD8+ T cells to stroke-damaged brain regions during the acute phase of stroke.
Methods: We examined age-associated neuroimmune signaling at acute, sub-acute, and chronic time points, utilizing an experimental pediatric stroke model in juvenile mice, compared to adult (middle cerebral artery occlusion, MCAO). We then performed immunohistochemistry, gene expression profiling, RNA-seq, and neurobehavioral testing.
Results: We find that increased CXCL10 levels in MCAO-injured juveniles result in recruitment of anti-inflammatory microglia and type 2 helper T cells (anti-inflammatory, Th2) towards the injury site, while in adults, proinflammatory microglia and CD4+ type 1 helper T cells (proinflammatory, Th1) and CD8+ cytotoxic T cells were present in damaged brain regions. Subsequent T cell activation further increases CXCL10 production and release in an anti-inflammatory feedforward loop in juveniles and proinflammatory in adults, which exacerbates tissue damage and motor impairments.
Conclusions: Our data shows compelling evidence that age-related differences in CXCL10 signaling results in anti-inflammatory responses in stroke-injured juveniles and proinflammatory in adults, demonstrating an instrumental role for TLR-mediated chemokine signaling and immune cell recruitment in neural regeneration and recovery.

Disclosures: K. Brown: None. **B. Akhter:** None. **M. Bradford:** None. **N. Aich:** None. **E. Williams:** None. **K. Rodgers:** None.

Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR386.25/J8

Topic: D.02. Ischemia

Support: AHA grant 24POST1198296
NIH grant NS122808

Title: Recurrent hypoglycemia exposure increases stroke risk by altering the platelet LIMK-cofilin pathway.

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Abstract: Stroke is a major complication in diabetes. Patients receiving antidiabetic therapy display an increased risk of recurrent hypoglycemia (RH). In an earlier study, we reported that RH exposure leads to increased stroke risk via platelet dysfunction. However, the underlying mechanism is not known. Earlier, we also observed that exposure to RH leads to cofilin hyperphosphorylation in platelets. LIM kinase (LIMK) phosphorylates cofilin, affecting actin dynamics, and is modulated by upstream regulators such as Rho p21-activated kinase 1 (PAK1) and (Ras homologus), while Slingshot phosphatase (SSH) activates cofilin by dephosphorylating it. We tested the hypothesis in the present study that RH-induced aberrant dysregulation of LIMK-cofilin pathway leads to platelet dysfunction via cofilin hyperphosphorylation. Male rats were rendered diabetic via a single intraperitoneal injection of streptozotocin and, 2 to 3 weeks later, treated with insulin pellet implantation (ITD). These animals were then subjected to RH. A control group (ITD + RH + Glucose) was maintained under euglycemia. Blood was collected ~24 hours after the last day of hypoglycemia and platelets were isolated. We observed that RH exposure results in a significant increase (163%) in LIMK activity (4.4 ± 0.9 nmol/hr, $n = 5$, $p < 0.05$) when compared to the ITD + RH + Glucose control group (1.7 ± 0.3 nmol/hr, $n = 7$). We next evaluated PAK1 and Rho kinase activity. RH exposure significantly increases PAK1 activity (4.51 ± 0.27 nmol/hr, $n = 5$, $p < 0.05$) by 49% as compared to the control group (3.02 ± 0.46 nmol/hr, $n = 5$). On the contrary, we did not observe any change in Rho kinase activity (data not shown). To investigate whether the rate of dephosphorylation of cofilin was altered, we next measured SSH activity. A non-significant decreasing trend (33%) in SSH activity was observed in the ITD + RH group (240 ± 49 pmol/hr, $n = 8$) compared to the ITD + RH + Glucose group (365 ± 55 pmol/hr, $n = 7$). Since cofilin regulates actin polymerization, we next evaluated the effect of RH exposure on actin polymerization. The actin polymerization was evaluated in terms of F/G actin ratio. The F/G actin ratio, when expressed as a percent of the control group, was significantly higher by 80% in RH-exposed ITD rats (180 ± 30, $n = 6$, $p<0.05$) compared to the control rats (100 ± 8, $n = 6$). These findings suggest that RH exposure leads to platelet dysfunction and induces cytoskeletal changes through LIMK-dependent cofilin hyperphosphorylation. Studies are underway to further characterize this pathway with a goal to identify a potential therapeutic target. Acknowledgment: AHA grant 24POST1198296 and NIH grant NS122808.

Disclosures: S. Mallepalli: A. Employment/Salary (full or part-time): full. **A.K. Rehni:** A. Employment/Salary (full or part-time): full. **S. Cho:** A. Employment/Salary (full or part-time): full. **K.R. Dave:** A. Employment/Salary (full or part-time): full.

Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR386.26/J9

Topic: D.02. Ischemia

Support: NIH Grant K08NS135129

Title: Microglial depletion ameliorates hippocampal dendritic spine loss in a mouse model of cardiac arrest with cardiopulmonary resuscitation

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Abstract: Background: There are over 350,000 cases of out-of-hospital cardiac arrest in the United States every year. Even with improved survival rates, long-term cognitive dysfunction remains common in survivors. The mechanism of ongoing synaptic dysfunction following global cerebral ischemia is not well understood, but effects on synaptic structures such as dendritic spines may play a role. Increasing evidence suggests that reactive microglia play an important role in modulating synapse architecture in the setting of brain injury through phagocytosis of synaptic material. The primary goal of this study is to elucidate the direct effect of reactive microglia on changes in dendritic spine numbers after a cardiac arrest brain injury. **Methods:** A murine cardiac arrest with cardiopulmonary resuscitation (CA/CPR) model was utilized to induce global cerebral ischemia in Thy1-GFP adult mice (age 8-12 weeks). To assess for changes in dendritic spine numbers after CA/CPR, brain tissue was collected at 72 hours and 7 days post procedure for spine analysis. To determine if microglia cells impact spine numbers after a CA/CPR injury, microglia levels in the brain were depleted with twice daily injections of the colony-stimulating factor 1 inhibitor PLX5622 beginning 72 hours post procedure and continuing until 6 days post procedure. Brain tissue was then collected for immunohistochemistry and dendritic spine density and microglia proliferation were measured and compared to sham animals. **Results:** Global cerebral ischemia in the setting of a CA/CPR injury resulted in a significant decrease in hippocampal secondary apical dendritic spine density 72 hours (1.7 versus 2.6 spines/µm, $p=0.006$) and 7 days (1.6 versus 2.0 spines/µm, $p=0.013$) post-injury in the hippocampus compared to sham animals. PLX5622 treatment significantly depleted hippocampal microglial numbers when given to mice that were subjected to either a sham or CA/CPR Injury. PLX5622 treatment increased hippocampal dendritic spine numbers in mice that survived a CA/CPR injury (1.44 versus 2.0 spines/µm in vehicle versus PLX5622 treated animals, $p=0.04$), while no change was observed with PLX5622 in the number of spines in mice that underwent a sham procedure. **Conclusions:** Our results demonstrate an effect of global cerebral ischemia on decreasing spine density at delayed time points post CA/CPR Injury, suggesting a decrease in post synaptic spine numbers may play a role in the long-term cognitive deficits experienced after ischemic brain injury. Additionally, the increase in spine density in mice with a CA/CPR injury treated with PLX5622 highlights that modulating microglia numbers impacts spine density.

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Poster

PSTR386: Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR386.27/J10

Topic: D.02. Ischemia

Support: DGAPA-PAPIIT IN214723

Title: Driving Axonal Regeneration: Neural Stem Cell Derived Extracellular Vesicles as a Repair Mechanism After Ischemia

Authors: *F. HERNÁNDEZ REAL[1], L. B. TOVAR Y ROMO[2];
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Abstract: Axonal damage is a hallmark of various neuropathologies, including ischemic stroke, where reduced blood flow induces morphological and functional alterations that precede neuronal death. Among the endogenous recovery mechanisms following stroke, neural stem cells (NSCs) play an important role, partly through the release of extracellular vesicles (EVs). These EVs transport proteins, lipids, and nucleic acids with regenerative potential. However, the specific contribution of NSC-derived EVs to axonal regeneration and neuronal survival remains insufficiently understood. In this work, neuronal viability, quantified by flow cytometry, and axonal regeneration, assessed with high-resolution microscopy using a PDMS[LBTYR1] -based microfluidic device, showed that EVs secreted by NSCs under normoxic conditions significantly enhance axonal regeneration and support neuronal survival of primary rat cortical neurons following ischemic injury. In contrast, EVs derived from ischemic conditions exhibit minimal regenerative effects. With the proteomic analysis of normoxic EVs, we identified the presence of the α-subunit of the coatomer complex (COPA), a critical component of Golgi-endoplasmic reticulum (ER) trafficking, as well as proteins associated with mitochondrial dynamics, both essential for neurite outgrowth and neuronal function. To determine the functional relevance of COPA, we depleted it in neuronal cultures and reintroduced it selectively via EVs. Our results show that while EVs from both normoxic and ischemic conditions maintain neuronal viability, only normoxic EVs promote axonal elongation and mitochondrial performance. These findings position NSC-derived EVs as a promising therapeutic strategy for delivering COPA and enhancing axonal regeneration after ischemic brain injury.

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Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.01/J11

Topic: D.04. Brain Injury and Trauma

Support: EY024481
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DM102446
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Title: Optic nerve injury activates an intercellular signaling pathway in mouse retina that promotes axon regeneration through serial activation of Kv2.1, GLT-1, NMDA receptors, and nitric oxide synthase

Authors: H.-Y. GILBERT[1], Y. LI[3], K. YUKI[2], K. OMURA[2], M. WALTER[4], N. HANOVICE[5], Y. YIN[6], P. HUANG[7], N. C. DANBOLT[8], Z. LIU[9], J. TANG[9], Q. ZHANG[9], E. AIZENMAN[10], L. I. BENOWITZ[11], ***P. A. ROSENBERG**[12,2];
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Abstract: Although retinal ganglion cells (RGCs), like most CNS neurons, normally cannot regenerate injured axons, they can be stimulated to do so by altering extrinsic as well as cell-autonomous signals. Cells use extracellular signals to coordinate responses to injury and to promote repair and regeneration. The NMDA receptor, which has a high affinity for glutamate and is functionally coupled to nitric oxide synthase, has the potential to mediate such responses by responding to changes in glutamate homeostasis. We report here that a multicellular signaling pathway in the mouse retina targeting the regulation of extracellular glutamate and nitric oxide production is activated upon optic nerve injury and modulates RGCs' ability to mount a robust regenerative response. A novel highly sensitive and specific NO sensor (FL2) revealed that optic nerve injury leads to a rapid, prolonged elevation of NO in the inner retina. Amacrine cell-specific genetic deletion of the neuron-specific isoform of nitric oxide synthase (NOS1, nNOS) or the NOS1 inhibitor N(w)-propyl-L-arginine (L-NPA) suppressed optic nerve regeneration. Steps leading to NOS1 activation are shown to include Kv2.1 phosphorylation and activation in RGCs, reversal of glutamate uptake by the glutamate transporter GLT-1 (EAAT2) in bipolar cells and glia, and subsequent NMDA receptor activation in amacrine cells. Conditional knockout of the glutamate transporter GLT-1 in bipolar cells, intraocular injection of the GLT-1 blockers dihydrokainate (DHK) or WAY213613, the N-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-5-phosphonovalerate (D-AP5), or the Kv2.1 blockers RY796 or stromatoxin all suppressed NO generation and strongly diminished RGCs' ability to respond to Pten deletion and other pro-regenerative factors. Thus, in the mouse retina after optic nerve injury, potassium channels in RGCs, glutamate transporters in neighboring cells, and NMDA receptors in amacrine cells are all involved in a pathway that activates the generation of nitric oxide and promotes axon regeneration after injury.

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Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.02/J12

Topic: D.04. Brain Injury and Trauma

Support: NIH Grant R01-EY029739

Title: Transcription Factor Regulation of Retinal Ganglion Cell Survival and Axon Regeneration After Optic Nerve Injury

Authors: *J. BRADY, A. LUKOMSKA, B. A. RHEAUME, E. F. TRAKHTENBERG; Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Retinal ganglion cells (RGCs) are prototypical central nervous system (CNS) projection neurons which transmit visual information to the brain. As other types of CNS projection neurons, RGCs fail to spontaneously regenerate axons after injury, resulting in permanent loss of vision due to conditions such as trauma, ischemia, and glaucoma. There are currently no approved clinical therapies which induce optic nerve/tract axon regeneration. The neuronal intrinsic factors which contribute to regeneration failure are still not fully understood, and while certain pre-clinical approaches targeting the proclivity of neurons to regenerate have been explored, these generally lead to sparse regeneration from a limited subset of RGCs. Here, we performed single-cell RNA sequencing (scRNA-seq) of RGCs following optic nerve crush (ONC) injury and treatments with knockdown (KD) of developmentally-upregulated experimental transcription factor 1 (Exp1-TF) and overexpression (OE) of developmentally-downregulated experimental transcription factor 2 (Exp2-TF), which we have identified as regulating RGC axon regeneration. Using bioinformatics analysis of the generated scRNA-seq datasets, we were able to determine the RGC subtypes which responded to these treatments. We also analyzed the downstream gene networks that are regulated by these treatments following optic nerve injury. We found partial overlap between the RGCs subtypes that responded to these treatments, and there was also a substantial overlap in the downstream gene networks associated with axon regeneration, which included several genes known to be involved in axon regeneration. Our findings provide new insights into the mechanisms of optic nerve axon regeneration, in an RGC subtype-specific manner.

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Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.03/J13

Topic: D.04. Brain Injury and Trauma

Title: Optic nerve regeneration requires the intracellular domain of LIFRα/CD118 but not LIF itself

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Abstract: Identifying receptors and ligands that enable retinal ganglion cells (RGCs) to regenerate injured axons is an important step in developing therapies to achieve functional recovery after optic nerve injury. Here we report that the intracellular domain of the leukemia inhibitory factor receptor (LIFR) is essential for RGCs' ability to regenerate injured axons independent of the cognate ligand (LIF) or other therapies. Overexpression of LIFR in adult RGCs is sufficient to induce neurite outgrowth in cultured RGCs and axon regeneration *in vivo*, while also amplifying RGCs' regenerative response to LIF itself and to several unrelated growth factors. Surprisingly, loss-of-function studies show that deleting LIFR eliminates the pro-regenerative effects of Pten deletion and of other established stimuli. LIFR was found to bidirectionally regulate the constitutive activity of the MAP kinase pathway, in contrast to LIF *per se*, which activates the pSTAT3 pathway. Moreover, expression of truncated LIFR lacking the extracellular domain is sufficient to promote axon regeneration and selectively increases the survival of particular RGC subtypes, placing the LIFR intracellular domain as a critical cell-autonomous regulator of optic nerve regeneration independent of ligand binding.

Disclosures: L. Xie: None. **L.I. Benowitz:** None.

Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.04/J14

Topic: D.06. Multiple Sclerosis and Other Demyelinating Diseases

Support: Hertie Foundation, Germany
FoRUM program of the Medical Faculty of Ruhr-University Bochum, Germany

Title: Divergent immune pathways in in EAE and OSE models of optic neuritis: transcriptomic insights into demyelination disorders

Authors: *S. C. JOACHIM[1], M. VALDES MICHEL[2], S. REINEHR[3], L. NEUMANN[4], J. ANDERS[2], K. KLÖSTER[4], R. KARDON[5], I. KLEITER[7,4], B. DICK[4], R. GOLD[8], S. FAISSNER[4], O. W. GRAMLICH[6];
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Abstract: Purpose: Optic neuritis (ON) is a frequent manifestation of autoimmune demyelinating disorders such as myelin oligodendrocyte glycoprotein-associated disease (MOGAD) and multiple sclerosis (MS). To study the underlying pathophysiology, various animal models are employed, including the induced experimental autoimmune encephalomyelitis (EAE) model and the spontaneous opticospinal encephalomyelitis (OSE) model. This study aimed to compare the pathomechanisms of EAE and OSE by transcriptomic analysis of the spinal cord and to relate molecular findings to ON phenotypes. **Methods:** EAE was induced in C57BL/6 female mice via immunization with MOG_{35-55} in complete Freund's adjuvant and pertussis toxin. For OSE, double-transgenic mice expressing MOG-specific T and B cells were used. Clinical scores were recorded daily, and retinal thickness was assessed by OCT on day 42. Spinal cords were collected for RNA sequencing, followed by differential expression analysis using DESeq2. Genes with adjusted $p<0.05$ and log2 fold change were considered differentially expressed. Pathway and gene ontology (GO) enrichment analyses were conducted using iPathwayGuide. Confirmatory RT-qPCR analysis were carried out. **Results:** Both EAE and OSE mice developed disability with a typical relapse followed by a remission phase and chronification in EAE and progressive disability accumulation in OSE. Retinal thickness was significantly reduced in both models. RNA sequencing revealed 639 differentially expressed genes out of 18,808. GO enrichment identified 2,067 significantly enriched terms, including regulation of cytokine production (p=6.19e-20), leukocyte activation (p=3.87e-15), and complement activation (p=7.35e-6) in EAE versus OSE. Pathway analysis indicated upregulation of the complement and coagulation cascades (p=1.52e-12), cytokine receptor interaction (p=1.49e-5), and hematopoietic cell lineage (p=2.06e-4) in EAE compared to OSE. Notably, CD14, C3ar1, and Serpina3n were significantly upregulated in EAE, while CD19 was downregulated compared to OSE. **Discussion:** The upregulation of CD14 and C3ar1 in EAE suggests a pathogenic mechanism involving monocyte activation and alternative complement pathway signaling, resembling features observed in MOGAD. In contrast, OSE mice showed increased expression of the B cell marker CD19 and transcriptomic signatures indicative of classical complement pathway activation, paralleling features of MS. These findings highlight distinct immunopathological signatures in EAE and OSE and their relevance for modeling ON in different autoimmune contexts.

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Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

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Program #/Poster #: PSTR387.05/J15

Topic: D.06. Multiple Sclerosis and Other Demyelinating Diseases

Support: JSPS KAKENHI Grant 24K18704

Title: Serotonergic system alleviates chronic pain in a rat model of Neuromyelitis Optica via 5-HT_7 Receptor

Authors: *T. ISHIKURA, T. ITOKAZU, T. YAMASHITA;
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Abstract: Chronic, treatment resistant neuropathic pain is a common symptom in neuromyelitis optica (NMO). Current therapies including antiepileptic drugs and opioids do not provide sufficient relief, and the underlying mechanisms of NMO neuropathic pain remain unclear. This study aims to elucidate the pathophysiological basis of chronic pain in NMO. We induced experimental autoimmune encephalomyelitis (EAE) in 8-week-old female Lewis rats by administering myelin basic protein, followed by injection of a pathogenic monoclonal anti-AQP4 antibody. Von Frey test revealed mechanical allodynia persisted up to 28 days post antibody administration. Histological analysis revealed prominent NMO lesions in the brain and spinal cord at the acute phase (4 days post antibody injection), which were no longer evident at the chronic phase (day 28). We focused on pain-related neural circuits, particularly descending pain modulation pathways. In human NMO, lesions often involve the regions in descending pain modulation pathways, such as the rostroventral medulla and spinal dorsal horn. Similar lesion distributions were observed in our rat model. Notably, we found increased serotonergic fiber density in the lumbar dorsal horn in the chronic phase. Intrathecal administration of 5,7-DHT, which ablates serotonergic neurons, exacerbated mechanical pain, and intrathecal selective serotonin reuptake inhibitor (SSRI) alleviated it suggesting an antinociceptive role of serotonin system in this model. Among various serotonin receptor antagonists tested, methiothepin (5-$HT_{1, 6, 7}$ receptor antagonist) induced hyperalgesia. Quantitative PCR analysis of the lumbar spinal cord revealed upregulation of 5-HT_7 receptor expression. Furthermore, administration of a 5-HT_7 receptor specific agonist alleviated pain, whereas its antagonist exacerbated it. These results suggest that 5-HT_7 receptor contributes to pain inhibition in NMO. Previous studies have shown that in models like spared nerve injury (SNI), 5-HT_7 receptors are expressed in GABAergic interneurons and exert analgesic effects. Additionally, compensatory 5-HT_7 receptor upregulation occurs in the context of GABAergic dysfunction. In our NMO rat model, immunohistochemistry confirmed increased 5-HT_7 receptor expression and decreased GABA expression in the spinal cord. Since GABA is synthesized from glutamate in astrocyte, we speculate that astrocyte dysfunction in NMO may underlie these serotonergic alterations. In conclusion, increased serotonergic innervation and upregulation of 5-HT_7 receptors in the lumbar spinal cord appear to play a compensatory, antinociceptive role in NMO-related chronic pain.

Disclosures: T. Ishikura: None. **T. Itokazu:** None. **T. Yamashita:** None.

Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.06/J16

Topic: D.06. Multiple Sclerosis and Other Demyelinating Diseases

Support: NSTC 112-2320-B-006-055-MY2
NCKUH-11210012
NCKUH-11310012

Title: Alteration of Proximal Signaling impairs Oligodendrocyte Development Following Neonatal Hypoxia Injury

Authors: *C.-Y. WANG;
Natl. Cheng Kung Univ., Tainan city, Taiwan

Abstract: Premature birth is often accompanied by immature cardiopulmonary function, resulting in complications such as hypoxia and increased oxidative stress in the developing brain. These factors markedly elevate the risk of neonatal brain injury, particularly hypoxic-ischemic encephalopathy (HIE), which leads to oligodendrocyte (OL) loss and impairs white matter development. Since OL maturation depends on complex intercellular cues—especially from neurons and astrocytes—hypoxic insults may disrupt this cellular communication, contributing to persistent white matter dysfunction. To investigate these altered interactions, we employed AAV-mediated split-TurboID proximity labeling combined with mass spectrometry to map protein-level interactions among neurons, astrocytes, and OLs after neonatal hypoxic injury. Integrating this approach with single-cell RNA sequencing and CellChat analysis, we identified a reduction in pleiotrophin (PTN) expression—a known OL-supportive growth factor—in astrocytes following hypoxia. Concurrently, expression of PTN's putative receptor, nucleolin (NCL), was diminished in hypoxic OLs. Functionally, astrocyte-conditioned media (CM) enriched with PTN enhanced OL maturation, while PTN deficiency further impaired OL survival and differentiation after hypoxic injury. These findings highlight a critical astrocyte-derived PTN-OL axis in white matter recovery and suggest that restoring intercellular signaling may offer a therapeutic strategy for neonatal white matter injury. Ongoing studies aim to uncover additional molecular pathways that support OL differentiation and myelination in the hypoxic neonatal brain.

Disclosures: C. Wang: None.

Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.07/K1

Topic: D.06. Multiple Sclerosis and Other Demyelinating Diseases

Support: NIH Grant R01NS124813

Title: Pikfyve loss in schwann cells impairs functional recovery after peripheral nerve injury

Authors: *B. HU;
Houston Methodist Res. Inst., Houston, TX

Abstract: Introduction: PIKfyve kinase, FIG4, and Vac14 form a PAS protein complex to catalyze production of PI3,5P2. Loss of function in either FIG4 or VAC14 causes de-/dysmyelination, but less is known about PIKfyve's role in the peripheral nerves. **Methods**: We developed a Schwann cell-specific *PIKfyve* knockout (*scPIKfyve*$^{-/-}$) mouse and employed nerve conduction study (NCS), morphometric analysis, and behavior assays to investigate whether PIKfyve is required for peripheral nerve functions. **Results**: There was no significant difference in nerve morphometrics, conduction velocity (CV), or compound muscle action potential (CMAP) amplitude between naive *scPIKfyve*$^{-/-}$ and *PIKfyve*$^{f/f}$ mice. After sciatic nerve crash injury, CV recovery followed a similar trend in both groups over a 32-week period. However, *scPIKfyve*$^{-/-}$ mice showed a significant decline in CMAP amplitude between 9 and 19 weeks post-injury ($P<0.05$). Gait analysis revealed a significant decrease in Stand Index ($P<0.05$) and Max Contact At % ($P<0.01$) of the hindpaws, and a significant increase in Maximum Contact Area and Print Area of the front paw on the injured side ($P<0.05$) in *scPIKfyve*$^{-/-}$ mice, compared with *PIKfyve*$^{f/f}$ mice. Hindlimb clasping response was significantly greater in the hindpaw of *scPIKfyve*$^{-/-}$ mice ($p<0.01$). **Conclusion**: Schwann cell *PIKfyve* ablation does not cause myelination abnormality, but results in functional impairments. Specific causes for these impaired functions will be further investigated at neuromuscular junction and spinal neuron body levels.

Disclosures: B. Hu: None.

Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.08/K2

Topic: D.04. Brain Injury and Trauma

Support: ANR-21-CE17-0019-01

Title: Translational biomarkers of hypoxic brain injury uncovered in CSF secreting human choroid plexus organoids

Authors: R. GASTON-BRETON, ***A. MABONDZO**, C. DISDIER;
CEA, DMTS, SPI, Neurovascular Unit Res. & Therapeut. Innovation Laboratory, Paris-Saclay University, Paris Saclay Univ., Gif sur yvette, France

Abstract: The choroid plexus-cerebrospinal fluid (ChP-CSF) barrier regulates a microenvironment supporting neural stem cell growth, strongly affected by hypoxia through ChP function. From human induced pluripotent stem cells (hiPSCs), here we established and validated *in vitro* ChP organoid secreting CSF-like fluid (iCSF) and exposed them to low oxygen atmosphere for 24h. Transcriptomic indicated major data on morphological and functional alterations in the ChP cells and shotgun proteomics revealed significant changes in proteins involved in energy metabolism and mitochondrial function. We found that H2AZ and ITM2B, involved in neurogenesis and neurite growth, were the key proteins downregulated in hypoxic iCSF and ChP organoids, respectively. Positive correlation analysis between hypoxia-induced mRNA expression of the neuronal progenitor biomarkers SOX2 and PAX6. Mature neuron MAP2 and H2AZ also confirmed impairment of neurogenesis. The results from this study suggest that ChP-CSF barrier opens new opportunities to characterize hypoxic brain pathophysiology and discover novel biomarkers.

Disclosures: A. Mabondzo: None. **C. Disdier:** None.

Poster

PSTR387: Brain Injury and Tissue Regeneration: Mechanisms and Therapeutic Targets

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR387.09/K3

Topic: D.06. Multiple Sclerosis and Other Demyelinating Diseases

Support: MWU One Health Research Award awarded to MT
MWU Start-Up Funds awarded to MT & ME
MWU Research Core Facility Outsourcing Funds awarded to MT

Title: Aging impairs recovery from peripheral neuropathy and hearing deficits after Schwann cell ablation in the DTA mouse model

Authors: M. EBEID[1,2,3], N. ZOGHBY[2], J. CRONBERG[2], A. SNAPKO[2], E. MARKUSON[2], S. SAWANT[2], S. LING[2], H. ZHANG[1], *M. TRAKA[1,2,3];
[1]Anat., Col. of Grad. Studies, Midwestern Univ., Downers Grove, IL; [2]Chicago Col. of Osteo. Medicine, Midwestern Univ., Downers Grove, IL; [3]Col. of Dent. Med. Illinois, Midwestern Univ., Downers Grove, IL

Abstract: The DTA mouse is an established animal model of specific cell ablation of *Plp1*-expressing glial cells and demyelination in the nervous system upon induction with tamoxifen administration (Traka et al., 2010). Previous work has shown that robust Schwann cell (SC) regeneration and axonal remyelination occur within a few weeks after SC ablation in the auditory nerve of young *PLP-CreER^T;ROSA26-eGFP-DTA* (DTA) mice (Wan & Corfas, 2017) and in

sciatic nerves of young-adult DTA mice (Traka et al., 2010). Here we leverage the DTA mouse model to investigate the process of glial cell regeneration and ultimately remyelination in auditory and sciatic nerves in the aging PNS. Three age groups of DTA mice were used: mature-adult (4-6 month-old), middle-aged (8-10 month-old) and aged (12-14 month-old) DTA mice were treated with 4-hydroxytamoxifen (4-OHT) to induce expression of the diphtheria-toxin subunit A and subsequent cell death in glial cells, whereas untreated littermates were used as controls. To analyze glial cell regeneration, recovering DTA mice of all three age groups are collected at different time points i.e. at 1, 3 & 5 weeks post-induction (w.p.i.) and their inner ears and sciatic nerves are fixed, then the auditory and sciatic nerves are dissected and immunostained either as whole mount or sectioned to label different types of glial cells, immune cells, as well as myelin and axonal markers. Transmission electron microscopy is used to identify myelin and neuronal ultrastructure. Auditory brain stem responses (ABRs) are used to assess hearing function in mature-adult and middle-aged mice. Grip strength testing is used to assess motor function in hindlimbs. We found that glial cell ablation is followed by a robust SC and satellite cell regeneration in the auditory system of the mature-adult DTA mice by 5 w.p.i., that relies mainly on cell proliferation. Nevertheless, ABR testing revealed progressive hearing deficits and grip strength testing showed muscle weakness in hindlimbs of mature-adult and middle-aged DTA mice, which also presented hindlimb clenching indicating Peripheral neuropathy (PN). Analysis of myelinated nerve fibers revealed disruption of the node of Ranvier domains in sciatic nerve, as well as increased numbers of macrophages and active ongoing demyelination with mild myelin loss in both sciatic and auditory nerve fibers of middle-aged DTA mice. Overall, our data indicate that despite the SC regeneration, persistent structural and functional defects exist in the PNS of aging DTA mice probably due to impaired SC remyelination.

Disclosures: M. Ebeid: None. **N. Zoghby:** None. **J. Cronberg:** None. **A. Snapko:** None. **E. Markuson:** None. **S. Sawant:** None. **S. Ling:** None. **H. Zhang:** None. **M. Traka:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.01/K4

Topic: D.05. Spinal Cord Injury and Plasticity

Title: A hypoxia conditioning paradigm for intra-uterine CNS programming

Authors: *F. C. DE BEER[1], H. W. STEINBUSCH[2];
[1]Maastricht University, The Netherlands, Zwolle, Netherlands; [2]Translational Neurosci., Maastricht Univ., Bunde, Netherlands

Abstract: A recent description of the rat's long primary afferent system development, with histological validation of our blueprint, has uncovered the unique potential of this new paradigm for intrauterine programming (doi.org/10.3390/anatomia2040032). Innovatively applied

microsurgery enables random exploration of the rat's long primary afferent system development and showcases fast and long-distance CNS regeneration restricted to the axons' critical period. The microsurgical dorsal myelotomy performed on fetal rats, which disrupts the development of the long primary afferent system, has been documented using axon track tracing. The arrangement of sequential steps creates a cascade in the DRG neuron development. These axon configurations exhibit features that are primarily determined by the intrinsic developmental plan and secondarily by an inferred, overriding factor that can promote rapid growth, i.e., regeneration.However, the ability to create unknown phenotypes, indicating intrauterine reprogramming, arises from hypoxia. Just thirty minutes of hypoxic conditions are sufficient to disrupt the original translatome. The susceptibility to hypoxia impedes the further development of the severed axons, even though they still possess the ability to regenerate into the medulla. Only the most upstream developing axons demonstrate this dynamic capacity confined to the system's critical period. Beyond this timeframe, severed axons fail to regenerate across the lesion site. Consequently, the gap between the upstream and downstream levels of fiber endings increases. A plausible explanation for an independent factor is a decrease in thrust. These unique sequelae, documented in the E15-E16 rat fetuses, are now visible to the naked eye. They indicate a narrow time window for the critical period upstream. Most likely, the decline in energy supply is the decisive condition, marking a watershed in temporo-spatial development. In addition to the existing criteria for CNS axon regeneration, axon elongation speed is an overlooked or unvalidated quality. In contrast, the slow formation of long-distance axon projections, as well as the ubiquitous capacity of short-distance collateral formation from mature axons, which is like that of developing neurons, all fail to meet the proper conditions for CNS regeneration. This undeniably differentiates the paradigm due to its intra-uterine programming ability, which overcomes the existing flaws in using neural cell cultures. These approaches may fall short due to the occurrence of adaptive translatomes, which happen to be masked by proteomic profiles, making them questionable in comparison to the paradigm's results.

Disclosures: F.C. de Beer: None. **H.W. Steinbusch:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.02/K5

Topic: D.05. Spinal Cord Injury and Plasticity

Title: Synthetic RNA-Mediated Enhancement of Functional Recovery Following Spinal Cord Injury Through Neuroepigenetic Modulation

Authors: *Y. LEE, E. JANG, Y. CHO;
Dept. of Brain Sciences, Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu Gyeongbuk, Korea, Republic of

Abstract: The restoration of protein synthesis is a critical component of axonal regeneration and a central objective in promoting central nervous system (CNS) regeneration. However, unlike the peripheral nervous system (PNS), which retains inherent regenerative capabilities, the CNS remains largely refractory to regeneration due to its inhibitory milieu. Overcoming this barrier remains a major challenge in regenerative neuroscience.In this study, we introduce a novel RNA-based therapeutic strategy utilizing a synthetic RNA element engineered from the 5′ untranslated region (5′UTR) of *Gpr151* mRNA. This engineered RNA (termed e5′UTR) engages with the RNA-binding protein CSDE1 to potentiate protein synthesis in injured neurons. Our in vivo results demonstrate that e5′UTR significantly enhances axonal regeneration and motor function recovery in both spinal cord and peripheral nerve injury models. These findings highlight the therapeutic potential of targeting CSDE1-mediated translational regulation as a neuroepigenetic intervention. Our approach establishes a versatile and non-genomic platform to modulate neuronal plasticity and advance RNA-based strategies for CNS regeneration.

Disclosures: Y. Lee: None. **E. Jang:** None. **Y. Cho:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.03/K6

Topic: D.05. Spinal Cord Injury and Plasticity

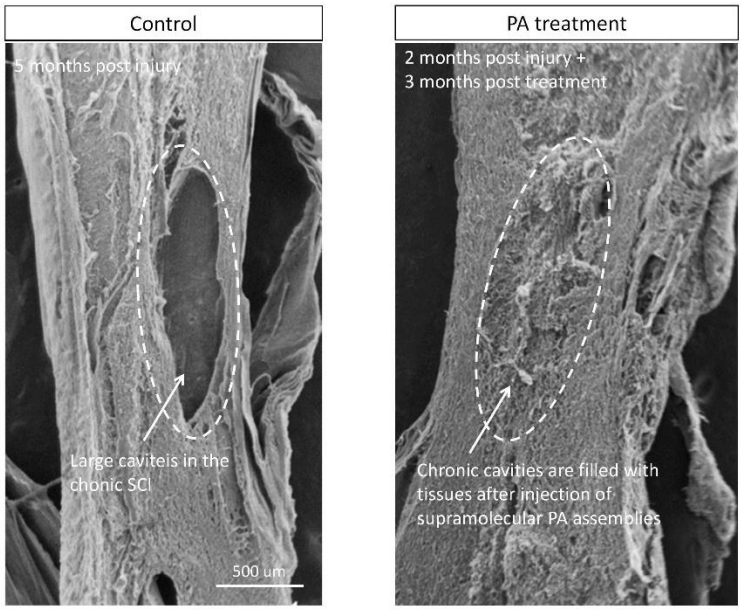
Support: A gift from the John Potocsnak Family for spinal cord injury research
Tito's foundation grant

Title: Bioactive Supramolecular Scaffolds Promote Neural Regrowth Within Cavities after Chronic Spinal Cord Injury

Authors: *Z. LI[1], G. SCESA[2,1], M. STRONG[1], Y. YU[1], A. METLUSHKO[1], N. TAKATA[1], N. SATHER[1], M. OUDEGA[2,1], S. STUPP[1];
[1]Northwestern Univ., Chicago, IL; [2]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Spinal cord injury (SCI) results in an untreatable loss of motor and sensory function. This lack of treatment leads to a steadily growing group of individuals with chronic SCI. Promoting axonal regeneration may support recovery of function in chronic SCI, but it is challenged by the presence of glial scar-encapsulated spinal cord cavities at the injury site. These cavities lack axon growth-supportive substrates and are surrounded by growth-inhibitory glial and fibrotic scar tissues. To promote axonal regeneration in the acutely injured spinal cord, we have utilized self-assembling peptide amphiphiles (PAs) containing laminin- and FGF2-mimetic peptides into the injury epicenter and found improved axon growth accompanied by improved locomotor function recovery (*Science 374: 848, 2021*). We have now extended our approach of using bioactive supramolecular assemblies as scaffolds to the challenging chronic injuries. For this purpose, we injected supramolecular PA assemblies into the lesion cavities of spinal cords

after 2 months of T10 contusion injury. Control rats received a saline injection into the cavities. Upon contact with cerebrospinal fluid, the PAs rapidly form nanofibrous scaffolds that mimic natural extracellular matrices (Figure 1). Interestingly, we found that ~86% of the cavity was filled with tissue after the PA injection, within which we observed serotonergic axon regrowth and more than a 3-fold increase in synapsin expression compared to the control group. In addition, PA-treated chronic contusion SCI rats exhibited significant recovery of hindlimb motor function after 3 months of injection; 6/12 PA-treated rats showed improved motor scores compared with 1/13 rats in the control group. These findings demonstrated the potential of our injectable bioactive supramolecular system for the treatment of chronic contusion SCI on its own or as part of future combinatiorial therapies.



Disclosures: Z. Li: None. **G. Scesa:** None. **M. Strong:** None. **Y. Yu:** None. **A. Metlushko:** None. **N. Takata:** None. **N. Sather:** None. **M. Oudega:** None. **S. Stupp:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.04/K7

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH grant NS047718

Title: Pten deletion in spinal pathways via retrograde transduction with aav-retro enhances hindlimb locomotor recovery after thoracic spinal cord injury but with accompanying impairment of bladder function

Authors: *N. NABI[1], O. STEWARD[2];
[1]Univ. of California, Irvine, Irvine, CA; [2]Reeve-Irvine Res. Centyer, Univ. of California Irvine, Irvine, CA

Abstract: Spinal cord injury (SCI) interrupts multiple different pathways causing loss of sensory, motor and autonomic functions. One strategy to deliver gene modifying constructs to multiple pathways interrupted by SCI is by injecting AAV-retrograde into the spinal cord. Previous studies have reported that AAV-rg carrying cargos that delete or knockdown PTEN enable some corticospinal tract (CST) regeneration accompanied by motor recovery, but with late-developing adverse effects. Here, we explore the approach further, focusing on locomotor and bladder function following severe thoracic injuries in mice. Adult male and female $PTEN^{f/f}$;$ROSA^{tdTomato}$ mice and $ROSA^{tdTomato}$ (tdT) controls received intraspinal AAV-rg/Cre injections at vertebral level T9 at the time of a severe T9 crush injury, resulting in retrograde PTEN deletion in neurons projecting to the spinal cord and also PTEN deletion at the injection site the spinal cord. Locomotor function was assessed using BBB scoring and bladder function was assessed by measuring the amount of retained urine at the time of daily bladder expression. $PTEN^{f/f}$;$ROSA^{tdT}$ mice that received AAV-rg/Cre (PTEN-deleted) exhibited greater recovery of BBB scores than $ROSA^{tdT}$ controls. However, bladder assessments revealed substantially higher levels of urine retention in $PTEN^{f/f}$;$ROSA^{tdT}$ mice than $ROSA^{tdT}$ controls indicating impaired bladder function. Stratification by sex revealed much higher levels of urine retention in males vs. females in both PTEN-deleted and control groups. To test whether injections of the AAV-rg/Cre would cause bladder deficits in the absence of PTEN deletion, $ROSA^{tdT}$ control mice received injections as above without concurrent SCI. Urine retention in uninjured $ROSA^{tdT}$ controls was minimal (under 0.1ml) with similar values in both males and females. These results indicate that bladder impairment was related to PTEN deletion and not the AAV-rg/Cre vector. To test whether bladder deficits were due to PTEN deletion at the spinal cord injection site or in retrogradely transduced neurons, $PTEN^{f/f}$;$ROSA^{tdT}$ mice and $ROSA^{tdT}$ controls received injections of non-retrograde AAV/Cre. PTEN deleted mice exhibited high levels of urine retention whereas urine retention in $ROSA^{tdT}$ controls was minimal with males exhibiting much higher levels of urine retention than females. Together, these findings indicate that AAV-based PTEN deletion in thoracic spinal cord circuitry can cause dramatic deficits in bladder emptying especially in male mice. Mechanisms for this impairment remain to be defined.

Disclosures: N. Nabi: 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, which is developing novel therapies for spinal cord injury and other neurological disorders..

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.05/K8

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Defense for Health Affairs, through the Spinal Cord Injury Research Program under Award #HT9425-24-1-0487

Title: Examining how norepinephrine hurts behavioral function after spinal cord injury: Evidence of a local effect at the site of injury.

Authors: *I. MORENO[1], T. JOHNSTON[2], C. T. PHINNEY[1], J. W. GRAU[1];
[1]Texas A&M Univ., College Station, TX; [2]McGovern Med. Sch. at UTHealth Houston, Houston, TX

Abstract: Following spinal cord injury (SCI), vasopressors such as norepinephrine (NE) are administered to fight the risk of fatal loss of blood pressure (hypotension). SCI can interrupt signals transmitted through the spinal cord leading to autonomic dysregulation. This causes blood pressure to deviate from the optimal blood pressure range, which has been correlated with worsened locomotor recovery. Physicians use NE to manage blood pressure, however recent studies have linked systemic NE administration and bouts of hypertension to increased tissue loss and poor recovery (Strain, 2021, J Neurotrauma, 38, 3406). Prior research from our laboratory has studied these effects using systemic drug administration (s.c. or i.v.). The current study explores the site of action, evaluating the impact of local application. Male rats received a contusion injury to the lower thoracic (T12) spinal cord. Experiment 1 was designed to replicate the finding that systemic NE treatment has an adverse effect. In addition, we examined whether the drug would affect performance of a spinal reflex (tail withdrawal from a noxious thermal stimulus). As previously reported, 0.5 mg/kg of NE (s.c.) produced increased systolic blood, a lasting disruption in locomotor function, and lowered spleen weight. Drug treatment also reduced reactivity to a noxious thermal stimulus (antinociception). Interestingly, the pain associated with tail-flick testing also undermined locomotor function. Experiment 2 evaluated the effect of local drug delivery. Immediately after the contusion injury, a catheter was secured for i.t. drug delivery. A day later, animals received 0, 15, or 60 nmol of NE dissolved in 10 µL of saline. Locomotor performance and cardiovascular function were assessed prior to drug delivery and hourly for 3 hours after. Local application of NE produced a transient increase in systolic blood pressure and an acute drop in locomotor function across the 3 hours of testing. Unlike systemic NE, i.t. drug treatment did not produce a decrease in spleen weight. Cellular assays are being conducted to assess hemorrhage, inflammation, and signals related to cell death. We are also examining whether local infusion of an alpha-1 adrenergic antagonist (prazosin) blocks the adverse effect of systemic NE.

Disclosures: I. Moreno: None. **T. Johnston:** None. **C.T. Phinney:** None. **J.W. Grau:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.06/K9

Topic: D.05. Spinal Cord Injury and Plasticity

Support: MNS137458A

Title: Differential Anesthesia Exposure and Neurological Outcomes in Spinal Cord Injury: Insights from the TRACK-SCI Database

Authors: *J. DAVIS, A. R. FERGUSON, J. HUIE;
UCSF, San Francisco, CA

Abstract: Patients with spinal cord injury (SCI) frequently undergo surgical and intensive care unit (ICU) interventions requiring anesthesia, yet the long-term neurological impacts of differential anesthesia exposure remain poorly understood. This study investigates the relationship between anesthesia exposure patterns in the operating room (OR) and ICU (intensive care unit) and neurological recovery, particularly locomotor function, as measured by the American Spinal Injury Association (ASIA) Impairment Scale. A retrospective cohort analysis was conducted using patient records from the TRACK-SCI database. Patients were stratified based on anesthesia type, duration, and cumulative exposure across surgical and ICU settings. Neurological outcomes were assessed using ASIA scores at admission and discharge time points. Multivariate analyses controlled for injury severity, demographics, and clinical confounders are underway. Preliminary findings suggest that differential exposure to certain anesthetic agents is associated with differential ASIA score trajectories. Further analysis is required to better understand the full extent of these effects. These findings highlight a potential role of perioperative anesthesia management in shaping neurological outcomes after SCI. Understanding how anesthesia exposure modulates recovery may inform tailored anesthetic strategies to optimize functional recovery in this population.

Disclosures: J. Davis: None. **A.R. Ferguson:** None. **J. Huie:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.07/K10

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NJCSCR Grant CSCR23ERG001
NIDILRR (RERC #90RE5021-01-00)
NIDILRR (ARRT #90ARHF0002)
the Tim Reynolds Foundation

Title: Assessment of Upper Extremity Kinematics and Muscle Activity in Individuals with Cervical Spinal Cord Injury Following Spinal Cord Transcutaneous Stimulation

Authors: *F. ZHANG[1,2], M. AGOSTINI[1], M. ANJARIA[1], A. BHEEMREDDY[1], M. RAVI[1], G. F. FORREST[1,2];
[1]Tim and Caroline Reynolds Ctr. for Spinal Stimulation, Kessler Fndn., West Orange, NJ; [2]Dept. of Physical Med. and Rehabil., Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: Restoration of upper extremity (UE) function remains a top rehabilitation priority for individuals with cervical spinal cord injury (SCI). Spinal cord transcutaneous stimulation (scTS) has shown great promise in neuromodulating spinal circuitry and facilitating function recovery following SCI. Quantitative kinematic and muscle activity analysis can provide valuable biomarkers for assessing the efficacy of scTS on movement patterns, joint coordination, and motor recovery. However, related studies are sparse so far. This study aims to investigate the changes in UE kinematics and muscle activation patterns following scTS combined with activity-based training (ABT) in individuals with cervical SCI. Participants with cervical SCI underwent 60 training sessions of scTS and ABT, with pre- and post-intervention handgrip, pinch, and modified functional reach testing. Sub-motor-threshold, tonic scTS (rectangular pulses with 1ms duration, filled with a carrier frequency of 5kHz) with optimized waveform, intensity, and frequency was delivered at the cervical and thoracic spinal segments over the dorsal skin while ABT was administered. ABT focused on repetitive unimanual activities of gross UE movement, grasping, and pinching without bilateral facilitation involved. Kinematic data were collected using a 3-D motion capture system during handgrip, pinch, and modified functional reach testing, while surface electromyography (EMG) recorded muscle activity from key UE and hand muscles (e.g. biceps brachii, triceps brachii, flexor carpi radialis, extensor carpi radialis, brachioradialis, extensor carpi ulnaris, flexor digitorum profundus, and opponens pollicis). Kinematic metrics included joint angles, spatiotemporal position, and range of motion. EMG amplitude and timing were analyzed to assess changes in muscle recruitment and coordination. Preliminary analysis revealed greater range of trunk motion and better trunk stability were achieved during modified functional reach testing after receiving scTS combined with ABT. Less compensatory movement and muscle coactivation were observed during handgrip and pinch strength post-testing, as compared to pre-intervention. Future studies should compare kinematics changes after scTS+ABT versus ABT only in a more heterogeneous group of individuals with diverse injury characteristics.

Disclosures: F. Zhang: None. **M. Agostini:** None. **M. Anjaria:** None. **A. Bheemreddy:** None. **M. Ravi:** None. **G.F. Forrest:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR388.08/K11

Topic: D.05. Spinal Cord Injury and Plasticity

Support: New Jersey Commission on Spinal Cord Research (CSCR23FEL002)
Tim Reynolds Foundation

Title: Transcutaneous spinal cord stimulation for cardiovascular regulation early after spinal cord injury. Is it safe, feasible and effective? preliminary findings

Authors: *E. ENGEL-HABER[1,2], A. BHEEMREDDY[1], M. B. BAYRAM[1,2], M. RAVI[1], H. SU[3], J. CARNAHAN[1], M. AGOSTINI[1], B. SNIDER[2,4], R. ZELENY[1], S. DILLON[1], A. STRUMOLO[1], D. PEPE[1], A. SCAGLIONE[1], S. KIRSHBLUM[1,2,4], G. F. FORREST[1,2];
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Abstract: Transcutaneous spinal cord stimulation (scTS) is a promising approach for improving cardiovascular (CV) regulation in individuals with spinal cord injury (SCI), particularly in managing blood pressure (BP) instability and orthostatic hypotension—common issues that hinder rehabilitation participation. While scTS has shown benefits in chronic SCI populations, evaluating its use in the early post-injury phase is crucial due to heightened neural plasticity and the onset of acute CV dysfunction. A key barrier to early intervention is conducting protocols in inpatient rehabilitation, where safety, feasibility, and preliminary efficacy must be carefully assessed. This study addresses that gap by evaluating the safety and feasibility of cardiovascular-targeted scTS (scTS-CV) in the subacute phase of SCI while exploring early signs of efficacy through systematic mapping and tilt-testing. We report findings from a clinical trial involving 6 participants enrolled within 50 days post-SCI during their inpatient rehabilitation. Each underwent baseline orthostatic tilt testing, followed by 2-3 days of spatiotemporal stimulation mapping to determine suitable stimulation parameters (amplitude, frequency, pulse-width, mono/biphasic waveform) and spinal segments (e.g., T7/8, T11/12, L1/2). Tilt table tests with sham and active stimulation were conducted while continuously monitoring BP and documenting adverse events. The study demonstrated that scTS (for CV and for broader applications) can be safely and feasibly implemented in the inpatient setting, with either no or minor study-related adverse events. Some participants experienced unrelated medical complications (e.g., urinary tract infections), requiring protocol adjustments. Despite logistical challenges and occasional scheduling delays, the intervention was mainly completed across participants with strong clinical and research team coordination. Preliminary findings illuminate cardiovascular response patterns during the critical early recovery period. The data suggest a temporally-defined phase of autonomic hyporesponsiveness, lasting approximately 1-2 months post-injury with inter-individual variation, during which both intrinsic autonomic function and stimulation responses are markedly reduced compared to chronic SCI cohorts. Enhanced responsiveness emerges following this refractory period. This work establishes the scientific foundation for larger-scale investigations to elucidate the chronological sequence of autonomic recovery and determine the optimal timing and efficacy of scTS interventions for CV regulation in acute and subacute SCI populations.

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Poster

PSTR388: Spinal Cord Injury: Recovery

Location: SDCC Halls B-H

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Program #/Poster #: PSTR388.09/K12

Topic: D.05. Spinal Cord Injury and Plasticity

Support: New Jersey NJCSCR #CSCR25ERG003

Title: Using muscle functional MRI to measure voluntary muscle activation in people with spinal cord injury

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Abstract: Several intervention modalities including locomotor training and neuromuscular stimulation have demonstrated the ability to promote neuromuscular recovery following both motor complete and motor incomplete spinal cord injury. While an array of methodologies and technologies exist for evaluating different aspects of recovery - for instance, stabilometry to assess balance and DEXA to assess bone mineral density - there remains a need for a means to comprehensively study spatial changes to muscle activation. Electromyography (EMG) provides temporal information for muscle firing during activity; however, it has several important limitations. While surface EMG (sEMG) is relatively easily collected, it cannot collect information on deep muscles and is susceptible to cross-talk - i.e. signals originating from muscles other than those intended to be studied. Fine wire intramuscular EMG is available but is invasive with a very limited recording area for each electrode, and both sEMG and fine wire EMG suffer from reliability issues related to sensitivity to exact placement of electrodes and changes in skin conductivity. In this project we are investigating the potential for muscle functional MRI (mfMRI) techniques to study changes in spatial patterns of neuromuscular activation associated with spinal cord injury (SCI). mfMRI imaging focuses on the time for relaxation of transverse magnetization (T2) of water in muscle, which has been found to be increased, i.e. prolonged, with exercise of the muscle. The phenomenon is understood to be related to an accumulation of osmolytes (phosphate, lactate, and sodium) in the muscle water. In this study, individuals with SCI and able bodied individuals perform supine quadriceps leg raise exercises against resistance provided by ankle weights on the detachable MRI table outside the scanner. Sessions start with several minutes of rest and baseline imaging to collect resting state T2, with exercise related T2 shifts calculated relative to this. Segmentation of the four quadriceps muscles on three image slices is used to isolate the activation distribution of each muscle; these are then combined to represent spatial activation patterns across muscles. We hypothesize that people with SCI have lower T2 shift values and less uniform muscle activation patterns than those observed across able bodied controls. The overall goal of this project is to provide new

information on spatial muscle activation patterns in SCI and produce knowledge to point the way to more widespread application of the mfMRI technique in this population.

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Poster

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Program #/Poster #: PSTR388.10/K13

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH NINDS Grant UH3NS116238

Title: Use of inertial sensors to predict loss of stepping independence while stepping with lumbosacral epidural spinal cord stimulation in individuals with motor complete SCI.

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Abstract: Previous studies have suggested that spinal cord epidural stimulation (scES) with locomotor training led to recovery of walking ability for individuals with spinal cord injury (SCI). However, participants still require various levels of assistance during stepping. There is limited past research to identify features that help predict a change of assistance during stepping with scES. Hence, we characterized changes across long independent stepping bouts and identified patterns that led to loss of independence using inertial measurement units (IMUs) in 3 individuals with motor complete SCI (AIS T2-T3). Resultant foot acceleration from foot IMU (a_{foot}) and sagittal plane angular velocity from shank IMU (ω_{shank}) were used to locate heel strike and toe off events and isolate stance and swing data. Independent stepping bouts of more than 25 continuous cycles were selected and separated into two 5-cycle segments after gaining independence, core central segment of >5 cycles, and two 5-cycle segments before losing independence. The features of interest were sagittal plane orientation (roll) of the foot and shank IMUs, along with ω_{shank}, and a_{foot}, and outcomes derived from them. The need of swing assistance after independent swing stepping bouts (482 bouts) were identified by the local maxima detected in the area under the $roll_{foot}$ curve, with an accuracy of 65.8%. In longer bouts with independent swing (58 bouts), cycle-to-cycle correlation of $roll_{foot}$, $roll_{shank}$, ω_{shank}, and a_{foot} was the highest in core phase (0.7-0.85), which decreased to 0.55-0.8 in last 5 cycles, and further to 0.3 to 0.7 when assistance was needed. When evaluating prolonged full left independent bouts, stance-to-stance correlation of $roll_{foot}$ and $roll_{shank}$ prior to and in the core phase (>0.94) was the highest, decreasing to 0.83-0.85 in the last 5 cycles, and further to 0.38-0.5 when they had to be assisted. However, these patterns had greater differences across the participants as compared to swing independence patterns. These results suggest that outcomes derived from inertial sensors

have the potential to identify changes in assistance patterns during stepping, especially during swing. In stance, the identification was inconsistent most likely due to a lower number of independent stance bouts than swing. The consistency could be improved by determining other composite variables that change closer to needing assistance. Further research on machine learning models that can help improve prediction is also warranted.

Disclosures: K. Joshi: None. **S.J. Harkema:** None. **M. Boakye:** None. **C.A. Angeli:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.11/K14

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Differential Brain Alteration and Their Relation to Sensory versus Motor Impairments in Chronic Cervical SCI Individuals

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Abstract: Spinal cord injury (SCI) disrupts the communication between the brain and the rest of the body, leading to a cascade of sensory, motor, autonomic, and cognitive impairments. SCI also triggers widespread reorganization and supraspinal changes that could explain the functional deficits. Magnetic resonance imaging (MRI) can be used to investigate the reorganization of neural circuits following SCI, offering insights into the neuroplastic changes both in term of anatomical and functional alterations. Data from 12 participants with complete and incomplete chronic SCI (1 female, 40.3 ± 18.7) and 12 age- and sex-matched able-bodied individuals (1 female, 48.2 ± 11.5) were acquired in the context of larger clinical studies that have received ethical approval from the institutional review board of Kessler Foundation. Anatomical (T1w) and resting-state (EPI) MRI data were acquired, and the SCI participants' arm and hand functions were assessed using the Graded Redefined Assessment of Strength Sensibility and Prehension (GRASSP). MRI data was analyzed using Cat12 and Conn Toolbox and gray matter volume and resting-state functional connectivity were measured and compared between groups and also while including clinical scores changes as covariates. Significant differences in cortical and subcortical regions volume and functional connectivity at rest were highlighted in

individuals with chronic cervical SCI as compared to the able-bodied individuals. Specific alterations include changes in resting-state functional connectivity of cortical and subcortical motor and sensory regions with visual and fronto-temporal areas. Some brain regions demonstrate additional differences with regards to only-motor versus sensory deficits in the SCI group. We show that motor and sensory deficits can be linked to differences in brain functional and structural reorganization in individuals with chronic cervical injury. Some of the regions with altered resting-state functional connectivity show also changes in cortical thickness and/or overall gray matter volume in the cervical SCI group. Brain MRI outcomes could thus be crucial to gain insights into specific deficits after an SCI and help personalize interventions based on specific therapeutic goals.

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Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.12/K15

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH -UH3NS116238

Title: Spinal cord epidural stimulation for improvement of bladder emptying in subacute SCI: A case study

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Abstract: Introduction: Improvement of bladder function remains a high priority for individuals with spinal cord injury. Electrical stimulation of sacral roots and peripheral nerves have been used previously in this population without widespread adoption. Spinal cord epidural stimulation (scES) has been demonstrated previously to improve motor and autonomic function in individuals with spinal cord injury. However, de novo bladder specific scES remains in its infancy. In the present case report we present detailed bladder function outcomes following 8 weeks of scES targeted for bladder function in an individual with a motor and sensory complete SCI. Methods: A 38-year old male, 6.5 months post injury with a T5 AIS-A classification was implanted with a spinal cord epidural stimulator. The 16-electrode array was implanted on the lumbosacral spinal cord between L3-Sacral spinal segments. The individual performed self-intermittent catheterization approximately 8 times within a 24-hour period and was not on active pharmacological intervention. After implantation, bladder mapping sessions during urodynamics studies (UDS) were performed once or twice a week focused on increasing bladder capacity and

voiding. During laboratory sessions, mapping for voiding targeted volitional detrusor contractions with simultaneous reduction in urethral resistance, while maintaining a stable blood pressure. Results: Voiding was successfully achieved in the laboratory with increased flow and efficiency. Initial mappings resulted in flow peaking at 4.2 +/-1.33 ml/s with a volume of 17.7+/-7.7 ml. Attempts were characterized by a weak flow. Flow and efficiency increased over a period of 2 sessions reaching Peak flow of 4.5 +/- 0.3 ml/s and a maximum volume of 39 ml (average 33.3+/- 3.8 ml) during a single attempt. The participant was able to reinitiate voiding following a short relaxation period enabling a total voiding volume of 166 ml in a single session. Blood pressure remained stable during the filling and voiding phases of the UDS. Conclusions: Noticeable improvements in bladder function were present when scES was used to target bladder emptying. Data from repeated UDS show sustained improvements over time, providing preliminary evidence for the benefit of scES in subacute SCI.

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Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.13/K16

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Investigating the nature of early EMG responses (<15ms) to transcutaneous spinal cord stimulation

Authors: *M. RAVI[1], M. B. BAYRAM[1,2], A. BHEEMREDDY[1], M. ANJARIA[1], G. F. FORREST[1,2];
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Abstract: Transcutaneous spinal cord stimulation (scTS) has been shown to be a highly promising, non-invasive therapy option for functional and systematic recovery after spinal cord injury (SCI). A challenge in applying scTS is in determining the location of cathode electrodes (spinal site) and the parameters of stimulation (Amplitude, Frequency, Pulse Width). One way to determine these is by 'spinal mapping' - a technique where researchers stimulate different spinal levels, at different parameters, and monitor EMG responses at relevant muscles. Based on the responses, optimal parameters and stimulation sites are then chosen. However, there is confusion as to whether the early responses (<15ms after the onset of stimulation pulse) contains physiological data or is merely a stimulation artifact as some researchers ignore them while others include them in their analyses. While existing literature suggests the presence of both artifact and genuine physiological signals, such as R1 potentials and M-waves, within this

window, a clear understanding of the precise nature of these early responses remains crucial for accurate clinical interpretation and therapeutic optimization. This study aims to investigate the characteristics of EMG responses observed within approximately 15ms after stimulation pulse in individuals with SCI and able-bodied controls. Various signal processing techniques will be applied to the EMG data to characterize the different components of these early responses - such as latency, amplitude, symmetry and waveform morphology. By analyzing these features - we aim to differentiate those that indicate a physiological origin versus those that are consistent with passive electrical artifact to provide a more definitive understanding of the composition of the early EMG responses after scTS. This study will provide critical insights into the nature of these early EMG responses, which is essential for optimizing stimulation protocols, and developing robust biomarkers for assessing neurological function and recovery in individuals with spinal cord injury.

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Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.14/L1

Topic: D.05. Spinal Cord Injury and Plasticity

Title: Mri-based 3d reconstruction of the spinal cord to optimize epidural stimulation paddle placement in spinal cord injury

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Abstract: Introduction: Spinal cord injury (SCI) results in paralysis of the lower extremity that severely affects walking ability and quality of life. Lumbosacral spinal cord epidural stimulation (scES) combined with activity-based training facilitates recovery of functional locomotor capabilities by activating clinically paralyzed muscles. Moreover, our recent work demonstrates that the motor rehabilitative effects are more pronounced when the scES implant covers a larger portion of the lumbosacral cord. To enable prediction and pre-planning of optimal scES paddle placement before implant surgery to obtain maximum rehabilitative effects, quantitative MRI image analysis may play a pivotal role. Such detailed MRI studies will enable the precise identification and visualization of several neuroanatomical structures essential for determining scES paddle placement over the cord. **Methods:** Fifty individuals diagnosed with chronic spinal cord injury (SCI) and enrolled in studies investigating the motor rehabilitative effects of activity-

based training combined with scES participated in the study. High-resolution axial and sagittal MRI scans (3 mm slices, no gaps) were obtained to locate and manually annotate key structures, including dorsal and ventral roots, spinal cord, and vertebrae. The locations of these structures were identified using RadiAnt DICOM Viewer and marked using Mango [Multi-image Analysis GUI] to generate a 3D model of the spinal cord. To determine the location of the ES paddle on the spinal cord, anterior-posterior and lateral X-ray images of the spinal cord were obtained. All annotations were performed by expert and trained analysts. **Results:** The developed annotation methodology enables the identification of different neuroanatomical structures required to generate a high-resolution 3D reconstruction of the spinal cord. Moreover, inter-rater reliability assessments reveal substantial agreement and consistency between raters while annotating different structures. The length of the lumbosacral enlargement, the end of the conus medullaris, the course of the spinal nerves, and their relationship with the vertebral bodies vary across individuals. **Conclusions:** This study establishes a reliable MRI-based annotation methodology for the detailed 3D reconstruction of the lumbosacral spinal cord in individuals with SCI. We aim to utilize the comprehensive dataset further to develop machine learning and deep learning-based medical imaging models that automatically generate 3D spinal cord models, thereby assisting in decision-making for scES paddle placement.

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Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.15/L2

Topic: D.05. Spinal Cord Injury and Plasticity

Support: The Reynolds Foundation

Title: Comparison of high carrier frequency stimulation waveforms generated by transcutaneous spinal stimulation devices

Authors: *M. B. BAYRAM[1,2], A. BHEEMREDDY[1], M. RAVI[1], M. ANJARIA[1], G. F. FORREST[1,2];
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Abstract: Transcutaneous spinal cord stimulation (scTS) is a non-invasive modality which has the potential to have similar positive therapeutical effects as the invasive (epidural) counterpart for increasing the excitability of spinal circuits and the restoration of various sensorimotor functions for people with spinal cord injury (SCI).During scTS, a current ranging between 1-100 Hz in frequency and an intensity of 5-150 mA is applied, with cathode electrodes placed at

vertebral levels and anode electrodes generally at bilateral clavicular or anterior superior iliac crests. This creates an induced electric field (e-field) along the spinal cord while the direct current flowing from anodes to cathode electrodes, shown to have therapeutical effects. An scTS stimulator has different controls to change the stimulation parameters to refine these effects, such as current, pulse duration, frequency and pulse waveform configuration. In addition to these standard parameters, some stimulators can also use carrier frequency overlay of 5-10 kHz during a much lower (e.g. 35 Hz) burst frequency to reduce the discomfort that could be caused by scTS. This high carrier frequency has shown some greater improvements along feasibility studies of scTS for upper extremity rehabilitation for people with SCI. Although the general practice is similar, current researchers are using different devices for scTS to create the e-field.The aim of this study is to benchmark and compare the waveform generated by several different devices currently being used in human stimulation research (verified in PubMed Central as of 4th of June 2025), with the same high carrier frequency stimulation parameters. The waveform is directly correlated with the e-field thus different devices should create the same to have a replicable outcome. Any discrepancy may lead to different research-based outcomes or even cause adverse events.

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Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.16/L3

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIDILRR
Reynolds Foundation

Title: Voluntary Movement with Spinal Stimulation

Authors: *G. F. FORREST[1], M. RAVI[2];
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Abstract: Neuromodulation for the recovery of lower extremity motor function has shown much progress for those living with severe paralysis after spinal cord injury using lumbosacral epidural stimulation, yet availability is very limited due to high costs and surgery required. Non-invasive transcutaneous stimulation of the human spinal could be more accessible if shown to have similar efficacy. This observational case study shows the potential of non-invasive spinal cord transcutaneous stimulation (scTS) for the recovery of voluntary leg movement even after a diagnosed sensory and motor complete spinal cord injury.

Disclosures: G.F. Forrest: None. **M. Ravi:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.17/L4

Topic: D.05. Spinal Cord Injury and Plasticity

Support: RERC

Title: Effect of motor response on blood pressure modulation during transcutaneous spinal cord stimulation in individuals with SCI

Authors: *A. BHEEMREDDY[1], E. ENGEL-HABER[1,2], F. ZHANG[1,2], M. B. BAYRAM[1,2], M. RAVI[1], M. ANJARIA[1], G. F. FORREST[1,2];
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[2]Physical Med. and Rehabil., Rutgers, Newark, NJ

Abstract: Transcutaneous spinal cord stimulation (scTS) has been shown to be an effective and non-invasive intervention for neurorehabilitation to improve motor function for people with spinal cord injury (SCI). Blood pressure (BP) instability is a common issue that SCI populations struggle with, and scTS has been shown to have a potentially positive effect on modulating BP. ScTS was applied systematically at various spinal levels ranging from cervical to lumbar sites, and electromyogram (EMG) was recorded at various upper limb, trunk, and lower limb muscles across multiple time points. Simultaneously, continuous non-invasive BP (systolic, diastolic, and heart rate) were recorded, and the data was synchronized with EMG recordings and stimulation. EMG and BP outcome data were analyzed to evaluate the effect of motor responses on the modulation of BP response. Time-series analysis and cross correlation for EMG activity and continuous BP were performed, and the results are presented.

Disclosures: A. Bheemreddy: None. **E. Engel-Haber:** None. **F. Zhang:** None. **M.B. Bayram:** None. **M. Ravi:** None. **M. Anjaria:** None. **G.F. Forrest:** None.

Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.18/L5

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Tim & Caroline Reynolds Foundation

Title: Neuromodulation and Seated Postural training leads to an increase in independent standing

Authors: *M. ANJARIA[1], A. BHEEMREDDY[1], M. RAVI[1], M. B. BAYRAM[1,2], G. F. FORREST[1,2];
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Abstract: Multi-site spinal cord transcutaneous stimulation (scTS) of cervical, thoracic, and lumbar spinal sites, combined with step training, has been shown to synergistically target an increase in motor pool activity, interlimb, intralimb coordination of the lower limbs (targeted), and dynamic and postural balance control of trunk during stepping and walking overground for individuals with incomplete spinal cord injury (SCI). The spinal stimulation allows for the activation of spinal structures at specific segments, with a wider or narrower focused configuration of rostral-caudal cathode/anode placement and, thus, a less or more focused current flow. The fields of stimulation allow for the selective recruitment of proximal and distal motor pools at low or higher intensities. In this study, we will evaluate the effect of multi-site rostro-caudal spinal stimulation combined with seated postural training on standing and stepping postural control for lower and upper extremity and trunk motor pools. Individuals with an incomplete SCI completed at least 40 sessions of scTS + seated balance activity-based training. Bilateral neuromuscular, kinematic, spasticity , and functional outcomes were evaluated at baseline and post-intervention. These data demonstrate that rostro-caudal sites of transcutaneous electrical spinal cord stimulation+activity-based training can differentially be used to activate increased motor pool activity and functional outcomes.

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Poster

PSTR388: Spinal Cord Injury: Recovery

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Program #/Poster #: PSTR388.19/L6

Topic: F.07. Rhythmic Pattern Generation

Support: The National Institutes of Health Roadmap Initiative, Office of Strategic Coordination – The Common Fund, Other Transactions, Stimulating Peripheral Activity to Relieve Conditions (SPARC) Program Award # OT2OD024898.

Title: Spinal Level-Specific Effects of Epidural Stimulation on the external urethral sphincter activation in Male Rats with Chronic Spinal Cord Injury

Authors: *D. MEDINA AGUINAGA[1], R. F. HOEY[3], K. ASCH[2], M. ALMOSAWI[2], I. HATTAB[2], C. HUBSCHER[4];
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Abstract: Recent preclinical spinal cord epidural stimulation (scES) studies in rats have demonstrated improvements in multiple lower urinary tract (LUT) functions, including storage, detrusor pressure, and emptying. Specific targets that may modulate activity of the bladder and external urethral sphincter (EUS) include the sacral parasympathetic nucleus (L5-S1), the somatic motor neurons controlling the EUS (L6-S1), the sympathetic nuclei (T13-L1), and the lumbar spinal coordinating center (LSCC, L3). Since EUS effects are found for all targeted regions, the urodynamic and electrophysiological records from previous experiments (chronic complete spinal cord-injured (SCI) male rats under urethane anesthesia) were compared to identify potential differences based upon spinal level of stimulation. At the L5-S1 level, scES elicited EUS electrical activity characterized by low-latency single-spike responses, which were associated with either a reduction in voiding efficiency or no meaningful change. Low-frequency (5 Hz and 10 Hz) with high-amplitude (300 µA) scES at the L3 level elicited high-latency, complex multi-spike EUS activity resembling the naturally occurring phasic bursting pattern. This pattern of activation significantly enhanced voiding efficiency and, in some rats, was also associated with the induction of ejaculation. High-frequency (45 Hz and 60 Hz) with high-amplitude (300 µA and 500 µA) scES at the T13-L1 level elicited low-latency single-spike responses, which were associated with a moderate increase in voiding efficiency as well as ejaculatory responses. These findings highlight the spinal level-specific effects of scES on EUS activity and LUT function in male rats with chronic complete SCI. Stimulation at L3 most effectively promoted coordinated EUS bursting and enhanced voiding efficiency, while stimulation at L5-S1 and T13-L1 primarily induced single-spike responses with limited or moderate improvements. The observation of ejaculatory responses at both L3 and T13-L1 levels suggests that scES can engage broader autonomic circuits beyond micturition. These insights inform the development of targeted neuromodulation strategies for restoring LUT and sexual function following SCI.

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Poster

PSTR389: Nociceptors and Pruitoceptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.01/L7

Topic: E.01. Somatosensation – Pain and Itch

Support: JSPS KAKENHI 25K12166
JSPS KAKENHI 22K09068

Title: A Novel SCN9A Point Mutation Reduces Pain Sensitivity through $Na_V1.7$ Gating Alterations in DRG Neurons

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Abstract: The voltage-gated sodium channel $Na_V1.7$ is encoded by the *SCN9A* gene. It is predominantly expressed in small-diameter dorsal root ganglion (DRG) neurons, where it modulates the action potential threshold in peripheral nociceptors . Importantly, loss-of-function (LOF) mutations in *SCN9A* cause congenital insensitivity to pain (CIP), underscoring the essential role of $Na_V1.7$ in nociceptive signaling. Although various LOF mutations have been reported, their precise mechanisms causing CIP remain unclear. In this study, we identified a novel LOF mutation, F382V, in a CIP patient and generated knock-in mice harboring the mutation to investigate its effects on pain sensitivity and underlying mechanisms. Experiments employed blinded, age-matched (8-12 weeks) wildtype and homozygous littermates of both sexes, and noxious stimulus cutoffs were applied to minimize tissue damage. In behavioral assays, homozygotes exhibited increased withdrawal thresholds to mechanical (von Frey test) and heat-evoked (hot-plate test) stimuli (both $p < 0.001$) compared to wildtype, without significant sex differences. Next, whole-cell patch-clamp recordings in small-diameter (≤30 µm) DRG neurons revealed that F382V raised the current threshold for action potential initiation ($p = 0.043$), caused a ~10 mV depolarizing shift in the half-activation voltage of the $Na_V1.7$ current ($p < 0.001$), and reduced maximal conductance density by ~8 pA/pF ($p = 0.006$). Immunocytochemical staining of dissociated DRG neurons showed comparable membrane expression of $Na_V1.7$ in mutant and wildtype cells, indicating that current reduction can be attributed to altered gating rather than abundance of $Na_V1.7$ channels. These gating alterations of $Na_V1.7$ link ex vivo channel dysfunction to elevated pain thresholds in vivo. Altogether, our findings provide a new insight into the physiological role of $Na_V1.7$ in pain signaling.

Disclosures: H. Nakamura: None. **T. Ishida:** None. **K. Ishida:** None. **S. Tanaka:** None. **M. Kawamata:** None.

Poster

PSTR389: Nociceptors and Pruitoceptors

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.02/L8

Topic: E.01. Somatosensation – Pain and Itch

Title: The role of extracellular vesicle-mediated communication in dorsal root ganglia on sensory neuron responses to pain signaling

Authors: *A. BISPO[1,2], K.-Z. DUAN[1], H. SHI[1], K. KISSNER[1], L. MACDONALD[1], D. C. SPRAY[2], N. ALESSANDRI-HABER[1];
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Abstract: Chronic pain is a multifactorial disorder, characterized by peripheral and central neuronal sensitization. First-order peripheral sensory neurons in the dorsal root ganglion (DRG) operate in a dynamic microenvironment controlled by cell-to-cell communication with neighboring neurons and non-neuronal cells. Cellular crosstalk within the DRG has been shown to modulate sensory neuron function and affect neuronal sensitization following injury and inflammation. Recently, the role of secreted extracellular vesicles (EVs) as mediators in the physiological mechanisms of pain signaling and pain chronicity has emerged; however, mechanisms and consequences remain poorly understood. Investigating EVs and associated cargo in the DRG could elucidate underlying mechanisms of pain and help identify novel targets for chronic pain treatment. We have taken several approaches to characterize DRG-derived EVs and to determine their impact on nociceptive sensory neuron phenotypes. We have isolated EVs secreted from dissociated DRG cultures following treatment with a cocktail of inflammatory mediators, an *in vitro* model of peripheral inflammation. Following the identification of secreted EVs by transmission electron microscopy and protein marker analysis, we evaluated the effect of released EVs on sensory neuron activity. Our calcium imaging and whole-cell patch clamp recordings from EV-treated sensory neuron cultures revealed that EVs secreted under inflammatory conditions in the DRG environment could functionally decrease neuronal excitability. Profiling of EV cargo through microRNA (miRNA) sequencing identified several miRNAs that were differentially packaged in inflammatory-stimulated EVs. We are now evaluating the effects of dysregulated miRNAs on sensory neuron activity and pain behavioral phenotypes. Our results suggest that EVs released locally in the DRG act to dampen neuronal excitability, potentially through miRNA-mediated gene silencing. Overall, this study provides insight into the role of EVs as communication mediators in the DRG, and the manipulation of EV associated cargo offers a novel potential tool for pain treatment.

Disclosures: A. Bispo: A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **K. Duan:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **H. Shi:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **K. Kissner:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **L. Macdonald:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals. **D.C. Spray:** None. **N. Alessandri-Haber:** A. Employment/Salary (full or part-time): Regeneron Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regeneron Pharmaceuticals.

Poster

PSTR389: Nociceptors and Pruitoceptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.03/L9

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R01 NS080889

Title: Role of corticosterone signaling in neonatal pain and reduced somatostatin expression in immature sensory neurons following early life stress

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Abstract: Traumatic childhood experiences, including sexual, physical, or emotional abuse and parental neglect, evoke severe early life stress (ELS). Our recently published data demonstrated that ELS acutely enhances mechanical and cold sensitivity in neonatal mice, accompanied by a reduction in somatostatin (*Sst*) mRNA expression in the neonatal mouse dorsal root ganglia (DRG). While peripheral Sst has been implicated in the tonic inhibition of nociceptive transmission in adults, the degree to which changes in peripheral Sst signaling may contribute to neonatal pain following ELS remains unclear. Furthermore, the underlying mechanisms by which ELS regulates *Sst* expression in the developing DRG have yet to be explored. Therefore, neonatal limited bedding (NLB) between postnatal days (P)2-P9 was used as a model of early life chronic stress, with litters housed under normal bedding conditions serving as a control. Experiments were then performed between P9-P10 to assess the short-term effects of ELS. The systemic administration of the stable, peripherally-restricted Sst analog, octreotide, reversed the mechanical and cold hypersensitivity caused by ELS, thereby suggesting that ELS may disrupt endogenous Sst-mediated antinociceptive signaling in the periphery. In addition, given that neuropeptide expression is known to be regulated by stress and glucocorticoids, we investigated the potential link between ELS-induced pain and canonical corticosterone (CORT)-mediated signaling. The data revealed that exogenous CORT administration during the early postnatal period was sufficient to evoke mechanical and cold pain hypersensitivity in neonates, as well as reduce *Sst* expression in the immature DRG, thereby mimicking the effects of the NLB protocol. As a result, we then sought to examine the potential role of glucocorticoid (GR) receptors in the ELS-induced plasticity within the DRG and emergent pain hypersensitivity. The conditional deletion of GR from immature, small-diameter sensory neurons (using *Scn10a-Cre* mice) did not alleviate the tactile, pressure, or noxious cold hypersensitivity evoked by ELS. Furthermore, *Sst* mRNA expression was downregulated in the DRG of both Cre-positive and Cre-negative mice subjected to the NLB paradigm. These findings demonstrate that GR signaling within sensory neurons is not required for the ELS-induced modulation of nociceptive processing or the downregulation of *Sst* expression within the neonatal DRG.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.04/L10

Topic: E.01. Somatosensation – Pain and Itch

Support: NS109059
DE018661
DE023090

Title: Enhanced excitability and mechanical sensitivity in trigeminal Aβ-Nociceptors contributes oxaliplatin-induced orofacial mechanical allodynia

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Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a major dose-limiting side effect of oxaliplatin treatment, often manifesting as debilitating neuropathic pain such as mechanical allodynia. While C- and Aδ-fibers were extensively studied for their potential involvement in neuropathic pain, the contribution of nociceptive Aβ-afferent neurons (Aβ-nociceptors) has not been well investigated. In the present study, we investigated whether trigeminal Aβ-nociceptors play a role in orofacial neuropathic pain, characterized by mechanical allodynia, following treatment with oxaliplatin. We combined behavioral, electrophysiological, and molecular biology approaches, utilizing Nav1.8Cre/YFP mice, a transgenic mouse line that allows for the identification of Aβ-nociceptors under a fluorescent microscope. Oxaliplatin-treated mice exhibited mechanical hypersensitivity in behavioral tests, as determined by the von Frey test and the AI-based measurement of orbital tightening. We then harvested trigeminal ganglion (TG) neurons from these animals and conducted electrophysiological recordings from trigeminal Aβ-nociceptors (Nav1.8-positive TG neurons). We found that in the oxaliplatin-treated group, trigeminal Aβ-nociceptors exhibited a significant reduction in AP rheobase and AP threshold, indicating increased excitability. Furthermore, voltage-clamp recordings revealed enhanced voltage-activated inward and outward currents in the oxaliplatin-treated group, further supporting a hyperexcitable state following oxaliplatin treatment. We further found that TTX-resistant sodium currents were significantly elevated, as demonstrated by electrophysiological recordings and also by RT-PCR, implicating Nav1.8 upregulation as a mechanism for the hyperexcitability of these neurons. Moreover, we demonstrate in the oxaliplatin treated group that mechanically activated currents were markedly increased in trigeminal Aβ-nociceptors, correlating with an upregulation of Piezo2 expression in the trigeminal Aβ-nociceptors. Our findings suggest that nociceptive Aβ-afferent neurons may be key contributors to oxaliplatin-induced mechanical allodynia, providing novel mechanistic insights into the pathophysiology of CIPN. These results highlight potential therapeutic targets for mitigating chemotherapy-induced neuropathic pain.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.05/L11

Topic: E.01. Somatosensation – Pain and Itch

Support: DE018661
DE023090
NS109059

Title: Effect of cooling temperatures on the intrinsic electrophysiological properties and ionic currents in Nav1.8-positive and Nav1.8-negative trigeminal Aβ-afferent neurons of mice

Authors: *A. SHARMA[1], S. GUPTA[2], J. GU[3];
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Abstract: Cooling temperatures profoundly affect somatic sensations such as touch and pain, with important clinical implications. Trigeminal Aβ-afferents can be classified as non-nociceptors, mediating touch, or nociceptors, mediating mechanical pain in the orofacial region and other head parts. Our previous studies using Nav1.8-ChR2 mice demonstrated that ChR2-eYFP$^-$ (Nav1.8$^-$) Aβ-afferents are predominantly non-nociceptors, whereas ChR2-eYFP$^+$ (Nav1.8$^+$) Aβ-afferents are primarily nociceptors. In the present study, we characterized the intrinsic electrophysiological properties and voltage-gated ionic currents of ChR2-eYFP$^+$ (Nav1.8$^+$) and ChR2-eYFP$^-$ (Nav1.8$^-$) trigeminal Aβ-afferent neurons and examined how these properties are affected by cooling temperatures. We observed significant differences between the two groups. Compared to ChR2-eYFP$^-$ (Nav1.8$^-$) neurons, ChR2-eYFP$^+$ (Nav1.8$^+$) neurons displayed more negative resting membrane potentials, lower input resistance, higher action potential (AP) rheobase and threshold, greater AP amplitude, and broader AP width. Voltage-activated Na^+ and K^+ currents were significantly larger in ChR2-eYFP$^+$ (Nav1.8$^+$) neurons. When temperature was reduced from 24 °C to 15 °C and 10 °C, ChR2-eYFP$^+$ (Nav1.8$^+$) neurons exhibited decreased conduction velocity, increased resting membrane potential and input resistance, reduced AP rheobase, and enhanced AP threshold and width. Although voltage-gated Na^+ and K^+ currents were suppressed by cooling, substantial current amplitudes persisted even at 10 °C. In contrast, while ChR2-eYFP$^-$ (Nav1.8$^-$) neurons showed similar changes to ChR2-eYFP$^+$ (Nav1.8$^+$) neurons at 15 °C, they failed to fire APs at 10 °C due to severe suppression of Na^+ currents. These findings suggest that Nav1.8$^+$ trigeminal Aβ-afferent nociceptors (ChR2-eYFP$^+$) retain the ability to transmit nociceptive signals at cooling temperatures as low as 10 °C, whereas Nav1.8$^-$ non-nociceptive Aβ-afferents (ChR2-eYFP$^-$) lose their excitability and fail to conduct innocuous sensory signals under the same conditions. Nav1.8 channels may thus play a critical role in enabling trigeminal Aβ-afferent nociceptors to resist cooling-induced functional suppression.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.06/L12

Topic: E.01. Somatosensation – Pain and Itch

Title: Neurotoxic chemotherapy enhances trigeminal sensory neuron sensitization in murine models of oral squamous cell carcinoma

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Abstract: Pain is one of the most common and debilitating symptoms in patients with cancer. While not all types are associated with pain, there are cancers for which pain is highly prevalent, including head and neck cancer (affecting 54-98% of patients). For these cancers, mutual cross-sensitization between cancer cells and peripheral sensory neurons can promote a highly pro-nociceptive environment that is also pro-tumorigenic. In head and neck cancers, tumor immunogenicity is directly linked to treatment resistance, impacting overall survival. Priming immune responses in the tumor microenvironment is promising strategy to improve treatment efficacy but may also impact sensory neurons innervating the tumor. Further, neurotoxic chemotherapies, including frontline treatments for head and neck cancer such as cisplatin, are also pro-nociceptive and induce painful peripheral neuropathy. Pro-inflammatory molecules released in the tumor microenvironment can directly sensitize nociceptors, and while targeting individual molecules and relevant receptors has been shown to decrease pain behaviors, nociceptor sensitization, and tumor growth, the composition of these molecules is not consistent for all tumors and treatment types, impeding the development of treatments for cancer-related pain that apply to a broad patient population. Treatments targeting nociceptor sensitization without hindering tumor immune activity, promoting cancer progression, or interfering with chemotherapy/radiation could provide more effective pain relief. Using oral squamous cell carcinoma cell lines, we conducted co-culture, conditioned media (CM), and orthotopic tumor studies to examine the impact of cisplatin on the electrical membrane properties of trigeminal ganglion (TG) neurons. We found hyperexcitability in small-sized TG neurons (putative nociceptors) of mice with oral squamous cell carcinoma (OSCC) tumors and in mouse TG neurons treated with OSCC CM. The addition of cisplatin to TGs cultured with OSCC CM enhanced neuronal sensitization, including medium-sized neurons that were not impacted under either condition alone, suggesting that distinct neuronal populations are involved. Further, mice bearing OSCC tongue tumors and treated with cisplatin also had enhanced TG neuron sensitization in *ex vivo* preparations. Inhibition of Nav1.8, which is preferentially expressed in nociceptors, blocked ectopic discharge after exposure to MOC1 CM. Identifying nociceptor subpopulations contributing to enhanced hyperexcitability in tumor-innervating TGs exposed to cisplatin could lead to more effective targets for pain relief in this patient population.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.07/L13

Topic: E.01. Somatosensation – Pain and Itch

Support: MSS (Korea) Grant 00509031

Title: Rci002, a novel trpv1 peptide antagonist, potently alleviates neuropathic pain without hyperthermia

Authors: *J. KIM[1], S. CHOI[1], Y. KIM[1], A.-J. LEE[1], H. KANG[2], H. JEON[2], Y. KIM[1,3]; [1]Rudacure Co., Ltd, Incheon, Yeonsu-gu, Korea, Republic of; [2]Rudacure Co., Ltd, Seoul, Korea, Republic of; [3]Col. of Med., Gachon Univ., Incheon, Korea, Republic of

Abstract: Neuropathic pain remains inadequately managed by current therapies, highlighting the need for safer and more effective analgesics. The Transient Receptor Potential Vanilloid 1 (TRPV1) ion channel is a well-known nociceptor involved in pain signaling. However, previous small-molecule TRPV1 antagonists failed clinically due to side effects such as hyperthermia, stemming from TRPV1's role in thermoregulation. Here, we present RCI002 - a distinctive peptide TRPV1 antagonist designed to overcome these limitations. We hypothesized that RCI002 would provide potent pain relief in neuropathic pain without the adverse effects seen with earlier TRPV1 inhibitors. To test this, we evaluated the efficiency and safety of RCI002 in both cultured sensory neurons and a rat model of chemotherapy-induced peripheral neuropathy (CIPN). In cultured dorsal root ganglion (DRG) neurons and TRPV1-overexpressing CHO cells, RCI002 effectively suppressed capsaicin-induced calcium influx in a concentration-dependent manner, confirming direct inhibition of TRPV1 activity. In a paclitaxel-induced rat CIPN model, the RCI002 treatment produced robust analgesic effects. Notably, RCI002 significantly reversed mechanical allodynia, as treated rats exhibited markedly higher paw withdrawal thresholds compared to vehicle-treated controls ($p<0.01$, 2-way ANOVA), approaching pre-neuropathy baseline levels. This pain-relieving effect was sustained for several days post-treatment with a gradual decline. Thermal hyperalgesia was also substantially attenuated following RCI002 administration. Importantly, RCI002 did not induce any observable side effects at therapeutic doses. Treated animals showed no changes in normal body temperature or motor function, unlike previous TRPV1 antagonists that typically cause hyperthermia. Our findings reveal that RCI002 potently alleviates neuropathic pain by selectively targeting TRPV1-mediated pain pathways while sparing normal thermoregulatory function. This novel mechanism enables significant analgesia without hyperthermic side effects. These results suggest that RCI002 offers a promising new therapeutic strategy for neuropathic pain, combining high efficacy with an improved safety profile as a peptide TRPV1 antagonist.

Disclosures: **J. Kim:** A. Employment/Salary (full or part-time): The author is an employee of RudaCure Co., Ltd, which may have a commercial interest in the research presented. **S. choi:** A. Employment/Salary (full or part-time): The author is an employee of RudaCure Co., Ltd, which may have a commercial interest in the research presented. **Y. Kim:** A. Employment/Salary (full or part-time): The author is an employee of RudaCure Co., Ltd, which may have a commercial interest in the research presented. **A. Lee:** A. Employment/Salary (full or part-time): The author is an employee of RudaCure Co., Ltd, which may have a commercial interest in the research presented. **H. Kang:** A. Employment/Salary (full or part-time): The author is an employee of RudaCure Co., Ltd, which may have a commercial interest in the research presented. **H. Jeon:** A. Employment/Salary (full or part-time): The author is an employee of RudaCure Co., Ltd, which may have a commercial interest in the research presented. **Y. Kim:** A. Employment/Salary (full or part-time): The author is the Chief Executive Officer (CEO) of RudaCure Co., Ltd, which which may have a commercial interest in the research presented..

Poster

PSTR389: Nociceptors and Pruitoceptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.08/L14

Topic: E.01. Somatosensation – Pain and Itch

Support: New York State Department of Health, Wadsworth Center, # C35596GG

Title: Development of chronic neuropathic pain following spinal cord injury in mice

Authors: K. GUNARATNA[1], H. LIU[2], ***M. PUOPOLO**[2];
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Abstract: Development of chronic neuropathic pain is a secondary and severe complication following spinal cord injury, affecting up to 60-70% of SCI patients. SCI-induced neuropathic pain (SCI-NP) is often lifelong and therapeutically intractable, leading to a severe decline in quality of life and increased risk for depression, anxiety, and addiction. Epidemiological studies addressing gender differences in chronic neuropathic pain in SCI patients have reported conflicting results, with some studies suggesting a tendency towards a higher pain and higher use of opiates and non-steroid anti-inflammatory drugs (NSAIDs) in women, others reporting no differences, and others reporting significantly higher prevalence in men. The aim of this work is to determine whether gender differences could play a role in the development/maintenance of SCI-NP in SCI mice. *Methods.* Wild type mice were used in this study. SCI was performed by a midline spinal cord contusion at T10 by using an Infinite Horizon Impactor (50 kilodynes, 1-s dwelling time). For behavioral experiments in vivo, the mechanical allodynia was measured with the von Frey filaments by using the up-down method with the 50% threshold. The conditioned place preference (CPP) paradigm was used to measure spontaneous pain. *Results.* In SCI male mice, the 50% mechanical threshold dropped from 1.48±0.09 g at baseline to 0.93±0.11 g

following SCI and to 0.95±0.08 g following vehicle injection. TTA-P2 (10 mg/kg, a blocker of T-type calcium channels) increased the 50% mechanical threshold to 1.46±0.11 g at 1-hour post-injection and to 1.11±0.13 g at 3-hour post-injection (n=12). For measurements of spontaneous pain, SCI male mice showed an increase in the TTA-P2-paired chamber of 59±35 s and a decrease in the vehicle-paired chamber of −76±31 s (n=12). In SCI female mice, the 50% mechanical threshold dropped from 1.38±0.08 g at baseline to 0.94±0.06 g following SCI and to 0.93±0.07 g following vehicle injection. TTA-P2 (10 mg/kg) increased the 50% mechanical threshold to 1.44±0.08 g at 1-hour post-injection and to 0.86±0.11 g at 3-hour post-injection (n=12). For measurements of spontaneous pain, SCI female mice showed an increase in the TTA-P2-paired chamber of 71±15 s and a decrease in the vehicle-paired chamber of −67±17 s (n=12). Data are reported as mean±SEM. *Conclusions.* Taken together, our data suggest that male and female SCI mice develop mechanical hypersensitivity and spontaneous pain to a comparable extend.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.09/L15

Topic: E.01. Somatosensation – Pain and Itch

Support: KQTD20200820113040070
JCYJ20220818100615033
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Title: C-fiber low threshold mechanoreceptor specific expression of TRPV4 negatively regulates mechanical pain and itch in hairy skin

Authors: *H. YAO[1,2], B. CAO[1,2], X.-J. SONG[1,2];
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Abstract: Transient receptor potential ion channels (TRPs) play essential roles in transmitting multiple sensory modalities, including pain and itch. Compared with well-studied TRPs (TRPV1, TRPA1 or TRPM8), TRPV4 is rarely touched, and most relevant studies focus on non-neuronal TRPV4 in regulating somatosensory. It is still controversial whether and how neuronal TRPV4 regulating pain and itch. C-fiber low threshold mechanoreceptors (C-LTMRs) represent a large population of unmyelinated sensory neurons that convey gentle and pleasant touch information. Some mouse and human genetic studies suggest that C-LTMRs may modulate nociception. However, it still remains elusive in the role of C-LTMRs in pain and itch sensation, and the molecular integrator for C-LTMRs' function has not been determined. In this study, we used highly specific and sensitive RNAScope to characterize *Trpv4* mRNA expression in different

classes of primary sensory neurons in dorsal root ganglion (DRG). In support of previous studies, *Trpv4* was expressed in small-diameter neurons and scarcely existed in myelinated A fiber neurons ($NF200^+$). Yet different from other studies, we identified that *Trpv4* was detected exclusively in TH^+ C-LTMR, but neither in $CGRP^+$ peptidergic neurons nor the IB4-binding neurons. Our data further confirmed specific expression of *Trpv4* in C-LTMR, but not in the *Trpv1*$^+$, *Sst*$^+$, *Mrgprd*$^+$, *Mrgpra3*$^+$ or *Hrh1*$^+$ nociceptor and/or prurioceptors. Ca^{2+} imaging of primary cultured sensory neurons showed specific Ca^{2+} response of C-LTMR activated by TRPV4 agonist (GSK101), which was diminished in C-LTMR *Trpv4* conditional knock-out animals (*Th*^{CreER}; *Trpv4*$^{fl/fl}$). Pharmacological perturbation of TRPV4 with GSK101 and QX-314 or genetic deletion of TRPV4 in C-LTMR enhanced mechanical pain and itch in the hairy skin, but not in the glabrous skin of naïve animals. These findings demonstrate C-LTMR specific expression of TRPV4 and suggest that C-LTMR negatively regulates mechanical pain and itch through TRPV4 in hairy skin in the physiological condition.

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Poster

PSTR389: Nociceptors and Pruitoceptors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.10/M1

Topic: E.01. Somatosensation – Pain and Itch

Support: Veterans Administration VISN4 Competitive Career Development Fund
FAER Mentored Research Training Grant
NIH 1R35GM150680

Title: Neuronal Ly86 mRNA is expressed primarily as a truncated transcript in nonpeptidergic sensory neurons and regulates inflammatory cytokine expression

Authors: *H. RAVULA[1], J. JONES[2,3], D. HOLLINGSHEAD[4], H. ASIF[5], K. M. ALBERS[2,3], M. RITTER[6];
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Abstract: Nonpeptidergic sensory neurons that innervate the skin have a key role in inflammatory pain and itch. To better understand the development and functional properties of nonpeptidergic neurons, neurturin (Nrtn), a glial cell line-derived neurotrophic growth factor that supports these neurons, was expressed under the Krt14 promoter in skin keratinocytes of mice (NrtnOE mice). To assess the impact of skin-derived Nrtn on sensory neuron properties, transcriptomic analysis of NrtnOE dorsal root ganglia (DRG) were conducted. Interestingly, a significant increase in the immune associated gene *Ly86* was measured. *Ly86* is translated to myeloid differentiation 1(MD-1), a protein associated with the innate immune response via the

toll-like receptor (TLR) activation on macrophages, dendritic cells, and B cells. In human, reduced *Ly86* mRNA expression is associated with proinflammatory disorders such as lupus and obesity. In addition, *Ly86*/MD-1 knockout mice have greater cardiac fibrosis in a model of hypertension. Together, these studies suggest that *Ly86* has an anti-inflammatory function. Therefore, we sought to understand the function of *Ly86* in nonpeptidergic neurons. In DRG of NrtnOE mice, we used RNAscope and RT-PCR to validate the increased level of *Ly86* mRNA in nonpeptidergic neurons. We found the full-length *Ly86* (exons 1-5) mRNA transcript in DRG neurons is produced at a significantly lower level compared to the spleen, which consists of immune cells. In addition to the full-length transcript, we also identified a highly abundant, Nrtn-regulated truncated *Ly86* mRNA transcript (comprised of exons 3-5), expressed in DRG but not detected in the spleen. This finding was further supported by RNA-seq analysis, which revealed strong exon 3-5 coverage and absence of signal from exons 1-2 in DRG neurons. However, no translated protein was identified for this truncated mRNA, raising the question of its role in neurons. To begin to examine the functional impact of neuronal *Ly86*, DRG neurons from wildtype and *Ly86* knockout mice (BAKO) were dissociated and cultured for 5 days in the presence and absence of Nrtn. Under these conditions, BAKO neurons expressed increased interleukin 1 beta (IL1-β) and tumor necrosis factor alpha (TNFα) in comparison to wildtype neurons, suggesting *Ly86* functions to suppress neuronal expression of inflammatory cytokines. Current studies are investigating mechanisms by which *Ly86* regulates neuronal cytokine expression.

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Poster

PSTR389: Nociceptors and Pruitoceptors

Location: SDCC Halls B-H

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Topic: E.01. Somatosensation – Pain and Itch

Support: NIH grant 1R01DK141890-01
NIH SPARC 5U01NS113868-02

Title: Intersectional chemogenetics reveals multiple DRG afferent pain pathways

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Abstract: Dorsal root ganglion (DRG) sensory afferents convey visceral and somatosensory information to the spinal cord. DRG afferents are heterogeneous with respect to receptor expression and neurotransmitter expression and this is critical to their diverse array of stimuli sensitivities and function. Many small- and medium-diameter neurons express the capsaicin

receptor TRPV1. Capsaicin activates these afferents, causing noxious sensations and nocifensive pain behaviors. Ablation of TRPV1-expressing neurons decreases pain evoked by thermal stimuli but not mechanical stimuli. Importantly TRPV1 is expressed in distinct DRG subsets: neurons that express neuropeptides such as Substance P (SP) (encoded by the Tac1 gene), and $IB4^+$ non-peptidergic neurons. Here, using a novel $TRPV1^{Flp}$ mouse in combination with $Tac1^{Cre}$, reporter mice (ai65f, ai9 and RC::FLTG) and AAV we mapped and modulated distinct nociceptive afferents. As expected the $TRPV1^{Flp}$-ai9 mice labeled small-diameter, capsaicin-sensitive DRG neurons. Comparative staining showed that many TRPV1+ neurons expressed either substance P or IB4. Surprisingly $Tac1^{Cre}$-ai9 mice labeled not just SP^+ neurons, but also many SP^-IB4^+ small neurons and SP^-IB4^- large neurons, suggesting the Tac1 gene is transiently active in most DRG neurons during development. To map DRG afferents based upon active TRPV1 and Tac1 expression in the adult $TRPV1^{Flp}Tac1^{Cre}$ mouse, we used DRG injections of adeno-associated viruses (AAV) that expressed mcherry under the control of both cre and flp recombinase. We found many $TRPV1^+Tac1^+$ neurons, many $TRPV1^-Tac1^+$ neurons and some $TRPV1^+Tac1^-$ neurons in the DRG, which labeled distinct spinal cord lamina. All labeled subsets projected fibers to the hind paw. We then injected intersectional AAV that expressed the activating chemogenetic DREAD hM3Dq into the DRG of $TRPV1^{Flp}Tac1^{Cre}$ mice, and selectively activated hind paw afferent subsets using intraplantar injections of clozapine-N-oxide (CNO). Activation of all three afferent subsets evoked flinching, indicative of pain. Surprisingly the flinching times were $TRPV1^-Tac1^+ > TRPV1^+Tac1^+ > TRPV1^+Tac1^-$ fibers. These data indicate multiple DRG afferent subsets can evoke nocifensive behaviors when selectively activated.

Disclosures: S. Kim: None. **M. Patil:** None. **S. Nair:** None. **S. Hadley:** None. **T. Taylor-Clark:** None.

Poster

PSTR389: Nociceptors and Pruitoceptors

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Topic: E.01. Somatosensation – Pain and Itch

Support: NIH U19NS130608
NIH R01NS11129
K00NS120636

Title: Nociceptive priming by cytokines of the IL-6 family in vitro sensitizes human sensory neurons to prostaglandin E2 stimulation

Authors: M. L. UHELSKI[1], M. HELEŠ[4], Y. LI[4], K. E. MCDONOUGH[2], H. ELAHI[4], ***P. M. DOUGHERTY**[3];
[1]Pain Med., [2]Neurosci., [3]Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [4]The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Neuroplasticity is a driving force behind the development of pathological pain states. In this process, an initial injury triggers molecular changes in peripheral nociceptors, leading to sensitization. While this initial sensitization may resolve with time, the introduction of a secondary, often subthreshold stimulus, will elicit a much stronger response than in naïve nociceptors, which may not respond or would only transiently respond. This secondary stimulus leads to persistent hypersensitivity, allowing for examination of hyperalgesic priming mechanisms. Priming injury can also be used to examine nociceptive priming *in vitro* after the primary stimulus is applied *in vivo*. While these experiments are feasible in rodent models, their use in patient populations are complicated by high variability in acute and chronic pain history and limited access to patient tissue. Thus, the current study aimed to determine whether the application of the primary stimulus *in vitro* could elicit nociceptive priming upon application of the secondary stimulus during patch clamp electrophysiology recordings. Two of our primary stimuli are cytokines that were selected based on their ability to elicit acute sensitization in cultured human DRG neurons, interleukin-6 (IL-6) and oncostatin M. These cytokines are both members of the IL-6 family and their receptors form signaling complexes with gp130. We also assessed leukemia inhibitory factor (LIF), another member of the IL-6 family that, like OSM, was found to be upregulated in male pain-associated DRGs in cancer patients with radicular neuropathy. These cytokines were applied to cell cultures from human DRGs collected from organ donors with no known history of chronic pain. Our secondary stimulus consisted of prostaglandin E_2 (PGE_2) at a low concentration—one that could be considered subthreshold as it failed to elicit prolonged sensitization in naïve neurons. Using patch clamp electrophysiology, untreated control and IL-6-, OSM-, or LIF-treated small-diameter DRG neurons were characterized prior to acute application of PGE_2 in current clamp. We found that incubation with IL-6, OSM, or LIF sensitized DRG neurons, as shown by an increased rate of ectopic action potential discharge, lower current thresholds, and increased responses to current stimulation. Primed neurons were also more sensitive to PGE_2, with more neurons responding to the secondary stimulus by firing action potentials or increasing the rate of ectopic discharge. Thus, *in vitro* priming with pro-inflammatory cytokines appears to be a valid method for studying nociceptor neuroplasticity in cultured human sensory neurons.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.13/M4

Topic: E.01. Somatosensation – Pain and Itch

Title: Frequency-dependent and frequency-independent conduction velocity slowing in peripheral axons of unmyelinated nociceptors in anesthetized mice

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Abstract: Partial inhibition of frequency-dependent slowing of conduction velocity—also known as activity-dependent slowing (ADS)—in C-fibres has been proposed as a hallmark of Nav1.8 channel blockade in non-human primates. As a first step toward evaluating this phenomenon in rodents, the present study investigated the fundamental characteristics of ADS in mice. Extracellular recordings from single unmyelinated nociceptors were obtained in the L4 dorsal root ganglion (DRG) of artificially ventilated, isoflurane-anesthetized mice. Baseline conduction velocity was measured at 0.2 Hz. Trains of 120 square-wave electrical pulses were applied at frequencies of 2, 4, 8, 16, and 32 Hz with an intensity of 2.5 times the activation threshold. Stimulation was delivered either at the receptive field on the paw or at the corresponding sciatic nerve branch (sural, peroneal, or tibial). Both receptive field and nerve stimulations were performed in each experiment. ADS was quantified as the percentage increase in latency relative to baseline latency for the final action potential in the train. Data are presented as mean±SD. Experiments were conducted on 11 mice. 1) ADS was obtained from one single-unit in all experiments. Conduction velocities measured from the receptive field were 0.77±0.09 m/s. 2) ADS was more pronounced with receptive field stimulations (e.g. at 2 Hz, 19.2±2.6 %) than with nerve stimulations (11.6±2.3 %). 3) ADS increased progressively with frequency from 2 to 8 Hz, reaching a plateau between 8 and 32 Hz. For receptive field stimulation, ADS at 8 and 32 Hz was 30.2±4.3 and 27.3±3.8 %, respectively; for nerve stimulation, 22.3±4.7 and 22.6±2.0 %. 4) A marked latency shift occurred between the 1st and 2nd pulse at 8, 16, and 32 Hz, with comparable magnitudes between receptive field and nerve stimulations. The "initial paired-pulse ADS" at 8, 16, and 32 Hz was 2.6±1.2 and 2.4±1.1%, 5.4±1.5 and 5.0±1.2 %, and 7.8±2.2 and 7.6±1.1%, respectively. When using standard ADS challenge in the present experimental conditions, final conduction velocity slowing was frequency-dependent below 8 Hz, and frequency-independent above 8 Hz. However, data obtained from the 1st and 2nd stimulations during ADS challenge revealed a frequency-dependent component decrease in conduction velocity at 8 Hz and beyond. These two forms of conduction slowing reflect distinct neurobiological mechanisms and offer complementary read out for pharmacological testing, which should not be restricted to Nav1.8 channel blockers.

Disclosures: J. Allard: None.

Poster

PSTR389: Nociceptors and Pruitoceptors

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR389.14/M5

Topic: E.01. Somatosensation – Pain and Itch

Support: Australian NHMRC

Title: 3d visualization of nerves in tissue cleared bone around human hand joints with osteoarthritis

Authors: *J. J. IVANUSIC[1], J. THAI[1], J. FULLER-JACKSON[1], E. EK[2], S. THAM[2], R. TEE[3]; [1]Anat. and Physiol., Univ. of Melbourne, Melbourne, Australia; [2]Melbourne Orthopaedic Group, Melbourne, Australia; [3]Univ. of Sydney, Sydney, Australia

Abstract: There is limited information available about the nerve supply to bones around human osteoarthritis (OA) hand joints to guide surgical approaches to pain management. In this study, we aimed to optimise a tissue clearing and light sheet microscopy protocol to visualize the 3D distribution of nerves in small hand bones of patients with advanced hand OA. Trapeziums, pisiforms and phalanges were obtained with written informed consent from patients undergoing excision of hand bones due to OA. Bones were immersion fixed overnight in 4% paraformaldehyde, then cleared using a modified version of our previously published protocol (Thai *et al.,* 2024). Axons and nerve terminal endings in bone were immunolabelled using an antibody directed against a pan-neuronal marker (protein gene product 9.5; PGP9.5). Samples were imaged using light sheet microscopy (Ultramicroscope Blaze, Miltenyi Biotec). 3D visualization and analyses were performed with Imaris (Bitplane). PGP9.5+ axons and nerve terminal endings were identified in all hand bones sampled. Nerve bundles followed blood vessels into each bone via nutrient foramina, branching extensively within trabecular bone and marrow cavity spaces. Many axons followed blood vessels, and had a corkscrew morphology typically associated with sympathetic innervation of the vasculature. Other axons ended away from blood vessels as free nerve endings, either near articular surfaces, or deep within the trabecular bone or marrow cavities. Some of these had *en passant* varicosities. No encapsulated endings were observed around axon terminals. Axons also projected towards articular surfaces through vascular channels in the subchondral bone, the greatest density of which occurred at articular surfaces where cartilage had degraded. Nerve entry points and distribution through each of the different bones varied. The pisiform was innervated by many small branches of one nerve that wrapped around the radial edge of the bone within a distinct bony groove. The innervation of the phalangeal head originated from nerves that entered the bone at the diaphysis, rather than the epiphysis. In the trapezium, many small nerve entry points were identified across the dorsal and volar aspects of the bone. This is the first detailed 3D visualization of the neuroanatomical distribution of nerves in intact human bone. It will directly inform the rational design of surgical approaches to pain management in advanced hand OA by clarifying where surgeons need to cut nerves, before they enter the small bones of the hand, for therapeutic benefit to be realised.
Reference: Thai *et al.*, 2024. *Journal of Comparative Neurology. 532(1): e25582*

Disclosures: J.J. Ivanusic: None. **J. Thai:** None. **J. Fuller-Jackson:** None.

Poster

PSTR389: Nociceptors and Pruitoceptors

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Topic: E.01. Somatosensation – Pain and Itch

Support: NIH NS136108
NIH NS045594

Title: Secreted frizzled-related protein 5 in satellite glial cells controls neuronal homeostasis and neuropathic pain

Authors: *A. PRUDENTE[1], W. XIE[2], J.-M. ZHANG[3], T. BERTA[1];
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Abstract: Neuropathic pain affects millions globally and involves complex interactions between glial cells and neurons. While glial cells can contribute to neuropathic pain by activating nociceptive neurons, they are also involved in maintaining neuronal homeostasis and promoting pain resolution. Satellite glial cells (SGCs), which envelop sensory neurons in the dorsal root ganglia (DRG), have emerged as homeostatic modulators of nociceptive function and potential therapeutic targets for pain resolution. Here, we identified and investigated the role of Secreted Frizzled-Related Protein 5 (SFRP5), a WNT signaling inhibitor, in SGCs and its contribution to maintaining neuronal homeostasis and alleviating neuropathic pain. Single-cell RNA sequencing across species revealed that SFRP5 is strongly expressed in SGCs, which was confirmed through fluorescent in situ hybridization co-localizing with SGC markers. SFRP5 is expressed around WNT5A-expressing neurons, suggesting an SGC-neuron paracrine signaling axis. In functional studies, targeting SGCs through intrathecal injection of SFRP5 neutralizing antibody and siRNA-mediated knockdown in naive mice led to mechanical hypersensitivity, suggesting SFRP5's role in nociceptive functions. Subsequent intrathecal administration of recombinant SFRP5 protein reversed the siRNA effect, confirming SFRP5's specific involvement. In vivo calcium imaging of DRG neurons showed increased spontaneous and stimulus-evoked activity after SFRP5 knockdown, demonstrating its role in maintaining neuronal homeostasis. To investigate SFRP5's role in neuropathic pain progression, we used a chemotherapy-induced peripheral neuropathy (CIPN) model created through multiple injections of paclitaxel, a common anticancer drug that causes temporary mechanical hypersensitivity. In this model, pain resolution correlated with increased SFRP5 expression in DRG tissues. Additionally, intrathecal injection of recombinant SFRP5 protein significantly reduced mechanical hypersensitivity. Overall, these findings demonstrate a crucial role for SFRP5 in SGCs in controlling neuronal homeostasis and CIPN. Further studies are needed to fully understand the SFRP5-WNT5A signaling pathway and determine if this therapeutic approach could help treat other types of neuropathic pain.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Title: An interorgan neuroimmune circuit promotes visceral hypersensitivity

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[1]Dermatol., [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Icahn Sch. of Med. at Mount Sinai, Larchmont, NY

Abstract: Visceral pain disorders, such as Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) and Irritable Bowel Syndrome (IBS) often manifest concurrently and overlap in symptom profiles. However, the mechanistic basis of such comorbidities and the transmission of neural hypersensitivity across organ systems has remained a mystery. Here, we identify a mast cell-sensory neuron circuit that initiates bladder inflammation and simultaneously propagates neural hypersensitivity to the colon in a murine model of IC/BPS. We unveil anatomic heterogeneity of mast cells in relation to nociceptors in the bladder and their critical dependence on Mas-related G protein-coupled receptor B2 (MrgprB2) to promote visceral hypersensitivity. Employing retrograde neuronal tracing, in vivo calcium imaging, and intersectional genetics, we uncover a population of polyorganic sensory neurons that simultaneously innervate multiple organs and exhibit functional convergence that drives bladder-colon sensitization. Importantly, using humanized mice, we demonstrate that pharmacological blockade of mast cell expressed MRGPRX2, the human ortholog of MrgprB2, attenuates both bladder pathology and colonic hypersensitivity. Our studies reveal evolutionarily conserved neuroimmune mechanisms by which immune cells can directly convey signals from one organ to another through sensory neurons, in the absence of physical proximity, representing a new therapeutic paradigm.

Disclosures: Z. Wang: None. **K. Song:** None. **B. Kim:** None.

Poster

PSTR389: Nociceptors and Pruitoceptors

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Topic: E.01. Somatosensation – Pain and Itch

Support: R01NS043314
5P20GM103418
Mabel A. Woodyard Fellowship

Title: Sensory Neuron Degeneration in a Tauopathy Mouse Model

Authors: *W. HAUSER[1], H. M. WILKINS[2], D. E. WRIGHT[3];
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Abstract: More than 20 million people in the US are suffering from some form of peripheral neuropathy. Damage to peripheral nerves can substantially impact the quality of life, and alterations in sensory neurons can contribute to the progression of numerous diseases. Peripheral nerve involvement in tauopathies is an essential non-motor symptom, as indicated by reductions in epidermal small fibers or intraepidermal nerve fiber density (IENFD) and incidences of pain, both of which are seen clinically. To understand mechanisms of pain and neuropathy in tauopathies, we began characterizing the sensory nervous system of the PS19 mouse model that harbors a P301S tau mutation, leading to an overexpression of the 4R tau isoform. So far, we have utilized methods of time-course-driven characterization of our model, particularly focusing on evoked and acute pain and cognitive behavior, IENFD, and inflammatory assays and staining in sensory DRG neurons. It is known that pathological tau deposits trigger an inflammatory response, activating toll-like receptor (TLR4) centrally, and we propose that pathological tau acts as a damage-associated molecular pattern (DAMP) and impairs PNS function via the TLR-NF-KB signaling pathway in peripheral neurons to drive an immune response, leading to comorbid small fiber neuropathy. Our data suggests that PS19 mice display paw thermal hypersensitivity at three months of age compared to Wild Type controls. At five months, PS19 mice display deficits in spatial memory, reduced paw IENFD, and activated microglia in the dorsal horn of the lumbar spinal cord. We have identified sex differences in thermal sensitivity. Ongoing studies aim to characterize glial neuron signaling specifically focused on TLRs as well as neuronal degeneration and responsiveness in vitro. Additional projects are focused on tau's interaction with sterile alpha and TIR motif containing 1 (SARM1), an enzyme that drives axon degeneration, and whether SARM1 plays a role in pathological tau-induced thermal hypersensitivity and IENFD loss. Knocking out SARM1 globally in PS19 mice prevented loss of IENFD, thermal hypersensitivity, cognitive deficit, and reduced expression of NMNAT2. We conclude that PS19 mice develop sensory neuron degeneration that parallel findings in tauopathy patients, and SARM1 is a potential target for preventing this comorbid phenotype. These studies aim to identify common mechanisms that may explain why peripheral nerve impairments are prevalent in tauopathies. Additionally, future studies aim to identify a link between chronic pain, inflammation, and cognitive decline, potentially offering predictive biomarkers or targets of intervention.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.18/M9

Topic: E.01. Somatosensation – Pain and Itch

Support: R35-NS127216

Title: Inhibition of the excitability of mouse sensory neurons by Kv1 channel enhancers

Authors: ***S. JO**, T. OSORNO, A. FUJITA, R. G. STEWART, B. P. BEAN;
Harvard Med. Sch., Boston, MA

Abstract: Kv1 family channels can strongly regulate neuronal excitability because they can be activated at subthreshold voltages. Kv1 channels are present in many primary sensory neurons and have been implicated in regulating pain sensitivity. Thus, pharmacological enhancement of Kv1 currents could be a promising strategy to suppress nociceptor excitability. Mouse nociceptors show higher expression of Kv1.6 than other sensory neurons (Zheng et al., 2019), suggesting that Kv1.6-containing channels could be a particularly promising target. To explore this possibility, we investigated compounds recently identified as enhancers of the Drosophila Shaker potassium channel (Silverå Ejneby et al., 2020). Our study aimed to address two key questions: (1) Can these compounds enhance human Kv1.6 (hKv1.6) currents? (2) Can enhancement of Kv1 currents effectively suppress nociceptor firing? To address the first question, we tested 14 of the Kv1-enhancing compounds (KVNs) for their effects on a human Kv1.6-expressing cell line, using the Qube 384 (Sophion Bioscience). Eight of the fourteen compounds tested at 30 μM significantly enhanced hKv1.6 currents and also slowed deactivation of the channels. We then performed dose-response assays with four of the most promising compounds. All four compounds increased tail currents at -40 mV following a step to -20 mV, even at 300 nM, and significantly prolonged tail current duration. To investigate the second question, we tested the functional effects of 1 μM KVN-006 on acutely dissociated mouse DRG neurons. Responses varied, likely reflecting the heterogeneity of DRG populations: K currents were enhanced in 6 of 12 neurons, unchanged in 3 neurons, and reduced in 3 neurons. Previous work by Sapio et al. (2020) reported high Kv1.6 expression in the nodose ganglion (NG). Accordingly, we also examined the effects of 1 μM KVN-006 on neurons from the acutely dissociated mouse jugular-nodose ganglion (JNG). K currents recorded at -10 mV or +10 mV were increased by KVN-006 in 14 of 16 neurons, reduced in 1 neuron, and unchanged in 1 neuron. To test whether KVN-006 enhancement was mediated via Kv1 channels, we co-applied 100 nM α-DTx in the continued presence of 1 μM KVN-006. All currents were subsequently blocked by α-DTx, confirming Kv1 channel involvement. 1 μM KVN-006 suppressed or abolished action potential firing in 8 of 11 JNG neurons, increased firing in 1 neuron, and had no effect in 2 neurons. These results suggest that pharmacological enhancement of Kv1.6 channels may be a promising strategy for reducing excitability of specific sensory neuron populations, offering a potential therapeutic avenue for the treatment of pain.

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Poster

PSTR389: Nociceptors and Pruitoceptors

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Program #/Poster #: PSTR389.19/M10

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH R35 NS127216
U19NS130608

Title: Kv1 channels powerfully regulate repetitive firing in capsaicin-sensitive human dorsal root ganglion neurons

Authors: *R. STEWART[1], K. NATARAJAN[2], A. FUJITA[3], T. OSORNO[4], S. JO[4], J. B. LESNAK[5], T. J. PRICE[6], B. P. BEAN[4];
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Abstract: Neuron firing patterns are regulated by the ion channels they express. The diversity of voltage-dependent potassium channels is particularly high relative to other voltage-dependent channels making their contributions to membrane excitability complex and varied. Kv1 subfamily channels are known to be highly effective "brakes" in controlling repetitive firing of some neurons because they can be activated by depolarizations below typical spike thresholds. Using whole cell patch clamp electrophysiology we investigated the role of Kv1 channels in controlling firing of human dorsal root ganglion neurons studied at 37°C. We found that currents from Kv1 channels are prominent in most small diameter neurons dissociated from the dorsal root ganglia of human donors that are capsaicin-sensitive and express Nav1.8 channels, likely corresponding to C-type nociceptors. In current clamp, the selective Kv1 inhibitor alpha-dendrotoxin enhanced firing frequency in almost all capsaicin-sensitive neurons and often produced a depolarization of resting potential as well as dramatic reduction in rheobase current, suggesting substantial steady-state activation of Kv1 channels at rest. Often, neurons that fired only one or two action potentials in response to long current injections in control fired repetitively after inhibition of Kv1 channels, with firing frequencies of up to 50 Hz seen in dendrotoxin. We characterized the kinetics and voltage-dependence of Kv1-mediated currents at physiological temperature using both voltage steps and action potential clamp experiments in which firing patterns were used as voltage commands. Kv1 channels often carried a large fraction of the total potassium current during the falling phase of the action potential. Most strikingly, Kv1 current accounted for almost all of the total outward current during interspike intervals, consistent with a dominant role in controlling repetitive firing frequency. The results show that Kv1 channels can strongly regulate excitability of capsaicin-sensitive human nociceptors and suggest that inhibiting or enhancing Kv1 channels might powerfully control human pain signaling.

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Poster

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Topic: E.01. Somatosensation – Pain and Itch

Support: R35-NS127216

Title: An automated patch clamp screen for enhancers of voltage-dependent potassium channels

Authors: *A. FUJITA, T. OSORNO, R. STEWART, S. JO, P. VAELLI, J. A. SMITH, J. SPLAINE, B. P. BEAN;
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Abstract: Some neurological disorders, including pain and epilepsy, can involve hyperexcitability of neurons. Therapeutic approaches to treat hyperexcitability have mostly been based on inhibitors of voltage dependent sodium channels. Another way of reducing hyperexcitability is to enhance currents carried by potassium channels, which are highly diverse among different types of neurons. Enhancement of Kv7 channels by retigabine and flupirtine is effective for treating some forms of epilepsy and pain, and enhancers have been found for a variety of other potassium channels, including BK and SK calcium-activated potassium channels, Kv1 channels, Kv3 channels, and K2P family channels. Some of these are under investigation for possible therapeutic development. To extend knowledge of compounds that may be promising for drug development, we carried out a screen for enhancers of three members of different Kv families, Kv1.6, Kv2.2, and Kv3.1b. A 25,000 compound library - *Ion Channels Focused* - from ChemDiv was screened on each of these channels, using whole-cell patch clamp recordings within a 384-channel automated patch clamp system (Sophion Qube 384). Use of patch clamp recording as the screen enabled detection of compounds that enhance currents by shifting the voltage dependence of activation or slowing deactivation, either of which are likely desirable features. To screen efficiently, we initially utilized a compound pooling strategy of testing 4 compounds in each well, with each compound present in 4 distinct wells, each time with different compound combinations. Compounds were applied at a concentration of 10 uM per compound. This strategy worked well for Kv3.1b, but failed for Kv1.6 and Kv2.2 because so many compounds inhibited rather than enhanced channel activity. Therefore, for these channels, we switched to a singlicate strategy of testing one compound per well and then re-tested compounds in which the well recording failed to meet quality control standards of current magnitude and stability before addition of compound. The results were very different for each of the channel types. Kv3.1b channels gave the largest number of hits for enhancers, followed by Kv1.6 channels. Interestingly, there were very few hits for enhancers of Kv2.2 channels, and even these gave only modest enhancement, while there were several compounds that produced complete inhibition of Kv2.2 channels at 10 uM. These results show the feasibility of identifying novel enhancers for Kv1.6 and Kv3.1b channels. On-going experiments to test the selectivity of the strongest enhancers among the channel families and determine activity on native channels in neurons will be discussed.

Disclosures: A. Fujita: None. **T. Osorno:** None. **R. Stewart:** None. **S. Jo:** None. **P. Vaelli:** None. **J.A. Smith:** None. **J. Splaine:** None. **B.P. Bean:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.01/M12

Topic: E.01. Somatosensation – Pain and Itch

Title: Characterization of cortical activity associated with tactile allodynia in patients with spinal cord injury: An EEG study.

Authors: *A. EDA, M. OSUMI;
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Abstract: Patients following spinal cord injury suffer from central neuropathic pain such as spontaneous and stimulus-evoked pain. Tactile allodynia is a type of stimulus-evoked pain which is characterized by pain elicited by normally innocuous stimuli such as light brushing of the skin.Although quantitative sensory testing (QST) includes the assessment of tactile allodynia in clinical settings, it relies on participants' subjective reports and lacks objectivity.Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have previously been used to quantitatively assess central neuropathic pain. Studies using these imaging techniques have reported that brushing stimuli capable of evoking pain activate multiple brain regions collectively referred to as the pain matrix, which are involved in pain perception and processing. However, both PET and fMRI require large-scale equipment and are not practical for use in routine clinical setting. In contrast, electroencephalography (EEG) is relatively simple and portable, making it more suitable for use in routine clinical settings. Therefore, in the present study, we aimed to clarify the characteristics of EEG activity associated with brush-evoked allodynia in individuals with central neuropathic pain.In this study, 26-channel EEG was recorded during brush stimulation in 31 healthy controls and 8 individuals with cervical spinal cord injury who exhibited brush-evoked allodynia.Time-frequency analysis revealed that the control group exhibited a significant event-related synchronization (ERS) in the beta band (16-30 Hz) between 350 and 700 ms after stimulation, predominantly over the bilateral frontal and central regions. In contrast, no significant ERS was observed in the patient group. A cluster-based permutation test confirmed that beta-band ERS between 350 and 700 ms after stimulation was significantly attenuated in the patient group compared to the control group. A positive linear correlation between β-band EEG activity and endogenous GABA concentrations has been reported, and the absence of β-band ERS in the patient group may reflect reduced cortical inhibition due to impaired GABAergic signaling. This absence of beta ERS may indicate reduced cortical inhibition and serve as a potential neural marker of tactile allodynia.

Disclosures: A. Eda: None. **M. Osumi:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.02/M13

Topic: E.01. Somatosensation – Pain and Itch

Title: Enkephalinergic neurons in the orbitofrontal cortex alleviate pain sensitization via local enkephalins release

Authors: *X. HOU[1], Y. LANXING[2], Z. ZHOU[2], G. WANG[3], Y.-Q. ZHANG[3];
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Abstract: Objective Activation of enkephalinergic neurons in spinal cord as well as subcortical structures shows analgesic effects. Yet now it remains not clear whether enkephalinergic neurons in the cortex has similar functions. A growing body of evidence demonstrates that neurons within the central nervous system demonstrate both coexistence and coordinated release of neurotransmitters and neuromodulators. However, the functional roles of neurotransmitters coexisting and co-released with enkephalins remain poorly understood. This study aims to elucidate the functional roles of enkephalins and coexisting neurotransmitters in enkephalinergic neurons of the cortex in mice. **Methods** (1) Transgenic reporter mice combined with histological techniques were employed to trace enkephalinergic neurons in the cortex. (2) Fluorescent in situ hybridization was to verify the co-expression of enkephalins and neurotransmitters. (3) A multidisciplinary approach encompassing viral injections, behavioral paradigms, pharmacology and whole-cell patch-clamp electrophysiology was implemented to investigate the functional roles of co-released enkephalins and neurotransmitters. **Results** (1) Enkephalinergic neurons are densely expressed in the orbitofrontal cortex (OFC) and predominantly localized to cortical layers II/III. (2) OFC enkephalinergic neurons co-express proenkephalin mRNA and vesicular glutamate transporter 1 mRNA. (3) Activation of OFC enkephalinergic neurons alleviates allodynia and depression-like behaviors induced by the chronic constriction injury of the sciatic nerve. (4) The analgesic effects are mediated through local enkephalin release acting on δ-opioid receptors and μ-opioid receptors within the OFC. **Conclusion** OFC enkephalinergic neurons mediate analgesic effects via local enkephalin release, while their antidepressive effects may involve glutamate release through extra-OFC axonal projections in mice.

Disclosures: X. Hou: None. **Y. LanXing:** None. **Z. Zhou:** None. **G. Wang:** None. **Y. Zhang:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.03/M14

Topic: E.01. Somatosensation – Pain and Itch

Title: Lysergic Acid Diethylamide (LSD) Decreases Pain Aversion

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Abstract: Pain is the most common chief complaint for hospital visits in the US. Further research into novel therapeutic options for pain is necessary because current medications have troublesome side effects or limited efficacy. Psychedelics such as lysergic acid diethylamide (LSD) are suggested to alter sensory experiences and could be a potential therapeutic in the setting of pain. However, the efficacy and mechanism of LSD particularly as a long-lasting analgesic remains unknown. Here we showed that a single dose of LSD can produce a persistent reduction in the aversive response to noxious stimuli and this anti-aversive property is mediated by prolonged suppression of the activity of neurons in the anterior cingulate cortex (ACC), a brain region well known to regulate pain affect. Firstly, in a classic conditioned place aversion (CPA) assay, we found rats showed avoidance for the chamber associated with mechanical pain. After intraperitoneal injection of LSD, however, this aversive response is no longer observed. Impressively, this effect lasted at least two weeks following the singular exposure to LSD. Further studies indicate that this anti-aversive effect was potentially mediated by the ACC as direct intracranial injections of LSD replicated these findings. Next, to evaluate LSD's effect on activity in the ACC we conducted neural recordings on free moving rats using Neuropixels probes. These experiments showed that LSD infusion resulted in a marked decrease in peak firing rate of ACC neurons, specifically those responsive to pain. Additionally, there was a drop off in the number of neurons responsive to pain following LSD administration. This decrease in firing was similarly present for up to two weeks following initial LSD exposure. These findings demonstrate an exciting and previously undiscovered role for LSD as a potential therapeutic for acute pain symptoms and expand our understanding of how this compound exerts its effect.

Disclosures: J. Plotkin: None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.04/M15

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R21TR004701

Title: Investigating the Molecular Impact of Transient Neuropathic Pain in the Mouse Frontal Cortex: A Whole Genome Methylation Sequencing Pilot Study

Authors: *A. KAUFFMAN[1,2,3], S. LEE[2,1], C. FAULK[4,1], L. S. STONE[2,1];
[2]Anesthesiol., [3]Comparative Mol. Biosci., [4]Animal Sci., [1]Univ. of Minnesota, Minneapolis, MN

Abstract: Chronic pain is often comorbid with affective disorders and is associated with neuroplastic changes in key brain regions including the prefrontal cortex (PFC). Epigenetic mechanisms such as DNA methylation are implicated in embedding long-term effects of pain and stress, altering how the organism might respond to subsequent challenge. We have previously shown that chronic neuropathic pain results in persistent changes in DNA methylation in the PFC for up to one year following injury. This research aims to determine if the methylation patterns in the PFC associated with nerve injury persist after the resolution of pain behaviors, offering critical insights into the long-term effects of injury and pain in the brain.We assessed behavioral and epigenetic changes following a sciatic nerve crush (crush) injury in adult female mice (C57BL/6J, 14 weeks). Mice were assigned to crush or sham surgery (n = 6 per group) and assessed for mechanical (von Frey) and cold (acetone) sensitivity, weight-bearing (black box), anxiety-like behavior (open field, elevated plus maze), and depression-like behavior (tail suspension, sucrose splash test) at baseline and weekly for 6 weeks. All behavioral analyses were conducted masked to group assignment, and sample sizes were determined *a priori* based on power analysis. Pain-like behaviors resolved by week 6 post-injury based on von Frey, Acetone, and Blackbox testing. Comorbid affective disorders were monitored, indicating a depression-like phenotype that also resolved by week 6. Anxiety-like behaviors were not detected. At this recovery timepoint, whole-genome methylation sequencing (WGMS) was performed on PFC tissue from representative sham and crush mice (n = 1/group) to screen for differential DNA methylation that persists after transient pain. WGMS revealed sites with at least 10% differential methylation in genes associated with neuroplasticity and synapse formation, glial activation and immune signaling, cell stress, and energy metabolism. These data support the hypothesis that pain experience leads to lasting epigenetic changes in the brain, even after behavioral recovery.

Disclosures: A. Kauffman: None. **S. Lee:** None. **C. Faulk:** None. **L.S. Stone:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.05/M16

Topic: E.01. Somatosensation – Pain and Itch

Support: UCI SSIHI Pilot Award
UCI Samueli Scholar Award

Title: The Analgesic Effect of Acupuncture is mediated by a Retrosplenial Cortex - Anterior Pretectal Nucleus Pathway

Authors: *H. ZHANG[1], W. CAO[2], C. NGUYEN[2], B. DE LA PEÑA[2], Z.-L. GUO[3], T. C. HOLMES[4], X. XU[2];
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Abstract: Acupuncture is an effective treatment for chronic pain conditions and is medically appealing as an inexpensive, non-addictive alternative with prolonged action. However, the neural circuit mechanisms underlying acupuncture analgesic effects remains elusive. In the present study, we measured the effect of EA on pain sensitivity in a mouse model of chronic inflammatory pain. EA application (2 Hz, 5 ms, 0.1-0.2 mA for 20 min) in the ST36 acupoint in the hindlimb, which is an acupoint frequently used for pain modulation, increases the mechanical pain threshold as measured by the von Frey filament test, indicating pain alleviation. The analgesic effect was confirmed by applying opto-acupuncture in ST36 in Prokr2-Cre:Ai32 mice that express channelrhodopsin-2 (ChR2) in the peripheral nerves in deep hindlimb fascia. To elucidate the neural basis of pain alleviation following acupuncture stimulation of ST36 EA, we mapped the brain activity with EA treatment in a TRAP2 mouse line that has tamoxifen-dependent, c-fos driven recombinase CreER, crossed with the Cre-dependent Ai9 reporter mouse. Our results show that EA stimulation of the ST36 acupoint significantly increases the numbers of tdTomato labeled neurons in multiple brain regions, including secondary motor cortex (M2), retrosplenial cortex (RSC), and anterior pretectal nucleus of thalamus (APT). *In vivo* calcium imaging indicates elevated activity of RSC excitatory neurons in response to EA stimulation of the ST36 acupoint. Similar results are observed with opto-acupuncture stimulation applied in Prokr2-Cre:Ai32 mice. Our results are consistent with previous studies reporting that RSC and APT contribute functionally to analgesic effects. We then used virus tools to identify the anatomic connection between RSC and APT. Projection terminals is observed in APT after injection of AAV-SynaptoTag in RSC, and injection of retroAAV-Cre in APT induces reporter gene expression in RSC, indicating a projection from RSC to APT. To examine the role of RSC-APT pathway in acupuncture effect, we injected retroAAV-Cre in APT and Cre dependent AAV-DIO-hM4Di in RSC, to target silencing APT-projecting RSC neurons. The analgesic effect of EA in inflammatory pain model is abolished when APT-projecting RSC neurons were chemogenetically inhibited. Using the same target activating strategy, chemogenetic activation of APT-projecting RSC neurons exerts analgesic effect in the inflammatory pain model. Our results indicate a critical role of RSC-APT pathway in EA mediated pain modulation.

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Poster

PSTR390: Cortical Pain Processing

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.06/N1

Topic: E.01. Somatosensation – Pain and Itch

Title: Cortical sensory perception and cognitive evaluation of painful stimuli after breathing-controlled electrical stimulation (BreEStim) - an EEG study

Authors: *A. RAO[1,2], M. HOUSTON[3,4], H. MENG[6,2], S. LI[6,2], Y. ZHANG[3,4,5], S. LI[6,2]; [1]UTHealth Houston, Houston, TX; [2]TIRR Mem. Hermann Hosp., Houston, TX; [3]Dept. of Biomed. Engin., Univ. of Miami, Coral Gables, FL; [4]Desai Sethi Urology Inst., [5]Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL; [6]Dept. of Physical Med. and Rehabil., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Breathing-controlled electrical stimulation (BreEStim) integrates voluntary breathing with transcutaneous electrical stimulation and has shown analgesic effects with accompanied central neuromodulatory effects. However, its underlying neural mechanisms remain unclear. In this study, we used electroencephalography (EEG) to investigate cortical changes associated with BreEStim, comparing it to standard electrical stimulation (EStim) to the left median nerve. Fourteen (14) healthy participants (7 M, 7 F) underwent both interventions, in random order with 3 or more days of washout. The interventions lasted 20-30 minutes depending on individual effort levels. The number of stimulations during intervention were matched for each participant across both conditions to ensure consistency. The pre- and post-intervention measurements included a 5-min resting-state EEG (64-channel) and electrical pain-threshold (EPT) level stimulations. EPTs were measured at the beginning of the experiment which were used to deliver 20 electrical stimulations to the contralateral median nerve at wrist in both pre- and post-intervention, while EEG data were recorded. The event-related potentials (ERPs) were analyzed for early somatosensory processing. Two-way ANOVA tests showed that there were no statistically significant differences in ERP amplitudes in the sensory cortex (CP3) at 20-40 ms ($p=0.5811$) after each intervention, suggesting no changes in cortical sensory perception. Resting state oscillatory activity of the left and right frontal and parietal lobes was quantified using normalized (post- /pre-intervention) power spectrum density (PSD) of the delta (1-4 Hz), theta (5-7 Hz), alpha (8-12 Hz), beta (13-30 Hz), and gamma (31-50 Hz) frequency bands and assessed with paired-sample T-test with a Benjamini-Hochberg adjustment. The delta band PSD was found to increase significantly only after BreEStim for the left ($p=0.0323$) and right ($p=0.0193$) frontal lobes as well as the left parietal lobe ($p=0.0314$). Frontal delta band power is associated with cognitive processing of affective aspects of painful stimuli. Taken together, our results of unchanged ERP amplitudes in the sensory cortex and increased delta power in the frontal lobes in the resting state EEG after BreEStim suggest that BreEStim does not alter cortical sensory perception but rather modulates cognitive processing of affective aspect of painful stimuli. These findings offer insights into the neurophysiological mechanisms mediating analgesic effects of BreEStim and may inform its therapeutic potential in neuromodulation.

Disclosures: A. Rao: None. **M. Houston:** None. **H. Meng:** None. **S. Li:** None. **Y. Zhang:** None. **S. Li:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

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Program #/Poster #: PSTR390.07/N2

Topic: E.01. Somatosensation – Pain and Itch

Support: 5F32AT012587
K99/R00-AT008238
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R21-AT010352

Title: Mindfulness meditation induced pain and stress relief is associated with weaker amygdala-perigenual anterior cingulate cortex connectivity

Authors: *J. G. DEAN[1], V. OLIVA[2], G. RIEGNER[1], F. ZEIDAN[3];
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[3]Anesthesiol., UC San Diego, La Jolla, CA

Abstract: Growing evidence indicates that stronger functional connectivity between the amygdala and perigenual anterior cingulate cortex (pgACC) mediates the robust relationship between pain and stress. Mindfulness meditation has the distinct capacity to alleviate pain and stress simultaneously. Yet, the brain mechanisms supporting the modulation of stress and pain by meditation remain poorly characterized. To bridge this gap, 40 pain-free and meditation-naïve volunteers (30±10 years of age; 20 males) were randomized to a four, 20-minute mindfulness meditation (n=20) or book-listening (n=20) intervention. Before training, noxious heat (ten, 10-second 49°C) was delivered (right calf) with a 16mm^2 thermal probe (TSA-II, Medoc). After training, the same noxious heat series was applied during both rest and meditation and functional magnetic resonance imaging (3T Siemens MAGNETOM: EPI; TE=25ms; TR=2000ms). Pain intensity was assessed after each heat series using a visual analog scale (VAS; 0 = "no pain," 10 = "worst pain imaginable"). Stress was rated pre- and post-MRI (VAS; 0 = "no stress," 10 = "worst stress imaginable"). Repeated-measures ANOVAs tested the hypothesis that meditation would produce greater pain and stress relief than rest and the control group. Psychophysiological-interaction (PPI) analyses modeled the convolved 49°C stimuli, the time series values from a bilateral amygdala seed (60% threshold; Harvard Oxford Atlas), and their interaction to determine if amygdala-pgACC connectivity predicted meditation-induced pain and stress relief. Within group regression analyses identified neural correlates of change in pain, stress, and their interaction ($z \pm 3.1$, $p<.05$). Mindfulness meditation significantly reduced noxious-heat induced pain by 32% ($p<.001$, $\eta p^2=.41$) and stress by 39% ($p=.02$, $\eta p^2=.13$) compared to rest and the control group. Weaker amygdala-pgACC connectivity during noxious heat moderated pain and stress reductions during meditation (interaction: $b=-0.103$, $p=.01$, conditional effect: $b=1.41$, $p=.002$). There were no significant amygdala-pgACC connectivity effects detected in the control group ($p>.05$). Mindfulness meditation-based pain and stress relief is moderated by weaker amygdala-pgACC connectivity, to potentially reflect decoupling between egocentric appraisals of noxious experiences.

Disclosures: J.G. Dean: None. **V. Oliva:** None. **G. Riegner:** None. **F. Zeidan:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.08/N3

Topic: E.01. Somatosensation – Pain and Itch

Title: Generalized pain onset detection from source-localized EEG using state-space modeling

Authors: *G. KENEFATI[1], M. ROCKHOLT[1], R. WU[1], H. MARTINEZ[1], Q. ZHANG[2], L. V. DOAN[1], Z. S. CHEN[3], J. WANG[2];
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Abstract: Chronic pain affects millions worldwide, yet objective biomarkers for pain detection remain elusive. In this study, we developed a state-space model (SSM) to detect pain state onset from source-localized EEG data. We analyzed EEG recordings from participants across three ongoing studies addressing chronic low back pain, chronic pancreatitis, and lupus. EEG data were source-localized to key pain-processing regions including dorsal and rostral anterior cingulate cortices (dACC, rACC), with spectral features extracted across key frequency bands (theta, alpha, low-gamma, high-gamma). Binary classification distinguished between low pain (ratings 0-1) and high pain trials (ratings 4+), with intermediate ratings excluded. The SSM framework achieved robust subject-level pain detection performance with F1-scores and ROC-AUC values exceeding 0.80. Low-gamma band activity (30-58.5 Hz) in bilateral dorsal ACC regions showed consistent performance across the patient population with F1-scores exceeding 0.60, emerging as a potential generalized biomarker for pain detection. These findings demonstrate the feasibility of real-time pain detection from EEG, providing a foundation for objective pain assessment tools in clinical and research applications.

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Poster

PSTR390: Cortical Pain Processing

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Program #/Poster #: PSTR390.09/N4

Topic: E.01. Somatosensation – Pain and Itch

Title: Cortical Signatures of Central Sensitization: TMS-EEG Responses to Thermal Pain in Human Primary Motor Cortex

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Abstract: Central sensitization, a mechanism underlying many chronic pain conditions, involves heightened responsiveness in central nervous system pathways. Understanding its early cortical signatures in healthy individuals can improve diagnostics and interventions. Transcranial magnetic stimulation combined with simultaneous electroencephalography (TMS-EEG) offers a non-invasive method to assess cortical excitability through TMS-evoked potentials (TEPs), yet the distinct responses to painful vs. non-painful thermal stimuli remain underexplored. We conducted a pilot study on two healthy adults (1 female, 1 male, ages 25-27) using neuronavigated single-pulse TMS over the left primary motor cortex (M1), targeting the right first dorsal interosseous (FDI) representation. Pain and warmth thresholds were individually determined using a Medoc TSA2 AIR thermode (baseline: 32°C, 1.5°C/sec). Three stimulation conditions were tested: baseline (no stimulation), acute thermal pain with continuous contact thermal pain adjusted to pain threshold, and noxious warmth (non-painful but intense). Each condition involved 100 pulses delivered at 90% rMT with inter-trial intervals of 4-6 s. EEG was recorded using a 64-channel cap, and TEPs were averaged over nine electrodes centered on left M1. Blinding was not applicable, but stimulus conditions were randomized to reduce order effects. In this pilot study, we compared cortical responses between a control (baseline) condition and a post-pain noxious warmth condition to capture early central sensitization dynamics. Compared to baseline, the warm condition showed significantly increased TEP amplitudes at N45 ($p < 0.01$), P60 ($p < 0.01$), and P180 ($p < 0.01$), indicating enhanced cortical excitability and prolonged sensory processing. Additionally, N100 showed a larger negative deflection in the warm condition ($p < 0.01$), a potential marker of altered GABAergic inhibitory function. These changes collectively reflect amplified cortical responsiveness consistent with central sensitization mechanisms following acute thermal pain. Collectively, these findings support the hypothesis that **acute pain triggers cortical excitability changes consistent with early-stage central sensitization**. Early peaks reflect disrupted excitation-inhibition balance, while later components may index altered salience and integrative processing. This paradigm may serve as a sensitive biomarker approach for characterizing pain responsiveness and maladaptive cortical plasticity.

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Poster

PSTR390: Cortical Pain Processing

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Program #/Poster #: PSTR390.10/N5

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant 1RF1NS132920-01A1

Title: Kappa opioid receptor signaling regulates activity of BLA inputs to L2/3 $mPFC^{Dyn+}$neurons

Authors: *A. E. O'SULLIVAN-DUFFY[1], H. A. TEJEDA[3], P. L. SHEETS[2];
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Abstract: Pain is a multidimensional experience with profound sensory and affective components. The medial prefrontal cortex (mPFC) is a key site for processing affective aspects of pain, yet the underlying circuit-level adaptations remain poorly defined. Dynorphin (Dyn), an endogenous opioid peptide, binds with high affinity to kappa opioid receptors (KORs) and is implicated in pain-induced aversion. The precursor peptide for Dyn, prodynorphin (Pdyn), is expressed in the mPFC of both humans and rodents, which includes expression in a subpopulation of neurons ($mPFC^{Dyn+}$). Administration of the Dyn into the mPFC elicits aversion in rats, making $mPFC^{Dyn+}$ neurons a potential substrate for regulating the negative valence associated with pain. Here, we used slice electrophysiology, optogenetics, and pharmacology to investigate how chronic neuropathic pain alters BLA-$mPFC^{Dyn+}$ synaptic function. To identify $mPFC^{Dyn+}$ neurons, we bred mice expressing Cre recombinase under the direction of the Pdyn promoter with Ai9-tdTomato to create Pdyn-tdTomato pups. In Pdyn-tdTomato mice (P60-90, both sexes), we injected pAAV-hSyn-hChR2(H134R)-EYFP (AAV1) into the basolateral amygdala (BLA) to allow for the selective optogenetic stimulation of BLA-mPFC projections. Whole-cell voltage-clamp recordings from fluorescently labeled $mPFC^{Dyn+}$ neurons revealed optogenetically evoked EPSCs (oEPSCs) representing BLA-driven excitatory presynaptic inputs. Bath application of a KOR agonist significantly reduced BLA-evoked oEPSCs in $mPFC^{Dyn+}$ neurons from sham mice, and this suppression persisted after washout, suggesting a presynaptic KOR-dependent form of long-term depression (LTD). Our initial recordings from spared nerve injury (SNI) mice at postoperative day 21 (POD21) indicate that KOR-mediated synaptic plasticity is maintained under chronic pain conditions. However, we find significant disruption in depolarization-induced suppression of excitation (DSE), indicating the possibility that retrograde postsynaptic regulation of presynaptic inputs is disrupted in chronic pain. Together, these early findings support a model in which chronic pain disrupts retrograde regulation of KOR+ BLA inputs to $mPFC^{Dyn+}$ neurons, potentially contributing to affective dysregulation.

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Poster

PSTR390: Cortical Pain Processing

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Program #/Poster #: PSTR390.11/N6

Topic: E.01. Somatosensation – Pain and Itch

Support: University of Iowa
University of South Florida

Title: High-frequency spinal cord stimulation reveals frequency-dependent modular organization of cortical networks in an ovine model

Authors: *V. BHARMAURIA[1], A. GHADERI[2], Y. B. BEZCHLIBNYK[1], N. SHAHEEN[3], K. JOHARI[4], A. SINGH[5], H. KAWASAKI[6], A. L. GREEN[7], H. OYA[6], A. M. LOZANO[8], M. A. HOWARD, III[6], O. FLOUTY[1];
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Abstract: High-frequency spinal cord stimulation (hSCS) is a promising therapeutic intervention for chronic pain, but its large-scale effects on cortical network organization remain incompletely understood. Previously, we demonstrated that hSCS suppresses the high-gamma envelope of evoked potentials in both somatosensory and association cortices, with mixed effects in the low-frequency range and inverse modulation patterns between these regions (Bharmauria et al., 2025, Society for Neuroscience). In this study, we examined how hSCS reshapes cortical network topology using 96-channel electrocorticography (ECoG) in sheep (n = 4). We computed frequency-specific functional connectivity matrices and applied Newman's modularity analysis to assess the organization of cortical modules before (TNS_0) and after (TNS_1) hSCS. Across animals, low- (4-12 Hz) and high-frequency (70-150 Hz) activity segregated into distinct modules that closely aligned with known anatomical boundaries between somatosensory and association cortices—despite the unsupervised nature of the analysis. Notably, while the somatosensory cortex maintained a relatively stable modular structure, the association cortex exhibited a pronounced decrease in modularity following hSCS, indicating a more fragmented and less coordinated network. Furthermore, intermodular trial-by-trial correlations revealed a frequency-dependent effect of hSCS: high-frequency networks displayed stronger and more dynamic cross-regional modulation compared with low-frequency networks, which remained more stable. Post-hSCS, high-frequency interactions between modules in the somatosensory and association cortices shifted markedly, often showing increased inverse correlations, suggesting an active rebalancing of cortical communication. These results indicate that hSCS induces a frequency- and region-specific reorganization of cortical networks, with high-frequency oscillations serving as sensitive markers of functional remodeling. Future work is targeted on inspecting the correlation in-depth and modeling the overall effect. Briefly, these findings highlight the capacity of modularity-based analysis to uncover physiologically meaningful network structure and suggest that cortical modular reconfiguration may underlie the therapeutic effects of hSCS.

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Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.12/N7

Topic: E.01. Somatosensation – Pain and Itch

Support: NIMH 1R01MH085849
NCI 1T32CA225590-01

Title: Parent-reported pain and cognitive problems linked to biomarkers and region-specific BOLD activity in adolescent survivors of pediatric acute lymphoblastic leukemia

Authors: *C. ARGENBRIGHT, J. C. TANNER, P. BELOATE, N. PHILLIPS, M. SCOGGINS, K. R. KRULL, T. BRINKMAN;
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Abstract: Introduction: Pain is a common late effect in survivors of childhood cancer, linked to treatment and poor functional outcomes. However, the biological mechanisms underlying pain and its impact on cognition remain poorly understood. This study examined links among pain, cognitive problems, biomarkers, and neural activity in survivors of acute lymphoblastic leukemia (ALL). **Methods:** Adolescent ALL survivors (N = 113) treated with chemotherapy-only assessed 5-10 years post-diagnosis (mean age = 14.36 years, SD = 4.56; 55.75% female) were evaluated for pain and cognitive problems via the parent-reported Pediatric Quality of Life Inventory Cancer Module. Serum was analyzed for markers of inflammation, oxidative stress, metabolic/cardiovascular function, and fibrosis/bone turnover. Resting state fMRI provided neural volatility measures within regions of the pain matrix. Associations among pain, cognitive problems, biomarkers, and neural activity were analyzed using multivariate and generalized linear models adjusted for age, sex, and time since diagnosis. **Results:** Parents reported pain in 27% and cognitive problems in 57% of survivors, defined as symptoms occurring 'sometimes' to 'almost always.' Joint pain was associated with worse cognitive problems (B = -5.43, p = .04), and higher frequency of severe pain was associated with lower levels of dehydroepiandrosterone sulfate (DHEAs; B = 0.80, p = .05), a neurosteroid involved in peripheral pain modulation. A joint pain and cognitive problems interaction was linked to DHEAs levels (B = 0.04, p = .01); survivors with more severe cognitive issues showed a stronger link between joint pain and lower DHEAs. Additionally, a pain status (yes/no) and cognitive problems interaction were linked to superior parietal lobe volatility (B = 0.04, p = .01); survivors with pain showed reduced activity, linking peripheral pain to cognitive processing. A pain status and malondialdehyde (MDA) levels interaction affected ventral anterior cingulate activity (B = 1.38, p = .05); higher MDA was linked to greater volatility, suggesting overlap between oxidative stress and affective pain modulation. **Conclusions:** Findings reveal a complex interplay among pain, cognitive problems, biomarkers, and neural activity in pediatric ALL survivors. Pain and cognitive issues were linked to adrenal neurosteroids, oxidative stress, and brain regions involved in cognitive and affective pain modulation. Results suggest endocrine and oxidative pathways may underlie pain-related cognitive challenges, highlighting the need to identify mechanistic biomarkers to guide interventions that improve both pain and neurocognitive outcomes.

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Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.13/N8

Topic: E.01. Somatosensation – Pain and Itch

Title: Quantitative Sensory Testing and EEG Reveal Cortical Dysregulation in SCD Pain

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Abstract: Pain in sickle cell disease (SCD) becomes increasingly complex over time, transitioning from acute to chronic states that affect central nervous system function. Yet, the cortical mechanisms driving this shift remain poorly understood. Neural markers such as alpha-band synchronization, linked to inhibitory control, and slow-wave reorganization have emerged as potential indicators of altered pain processing in chronic conditions. In particular, alpha hyper-synchronization—reflecting excessive or dysregulated rhythmic activity—may signal maladaptive cortical responses to persistent nociceptive input. Identifying such EEG-derived features in SCD could offer insight into sensory dysfunction and its neural correlates, but has not been reported. We investigated cortical mechanisms of altered pain processing in SCD using quantitative sensory testing (QST) and two EEG measures: event-related synchronization/desynchronization (ERS/ERD), capturing transient band-specific power changes, and canonical correlation analysis (CCA), mapping stimulus-linked network activity. Eight adults (4 with SCD, 4 pain-free controls) underwent QST to assess thermal detection and pain thresholds. EEG was recorded from a 32-channel system at 5000 Hz, down-sampled to 1000 Hz, band-pass filtered (1-50 Hz), and cleaned via independent component analysis. ERS/ERD, computed using Welch's method, quantified percent power changes from rest to assess hypersynchronization. Network-level cortical activity was analyzed using unidirectional CCA, projecting EEG features onto stimulus timing.
QST thresholds revealed SCD exhibited delayed sensory sensitivity and detection of thermal changes, yet reported earlier pain onset, which may reflect compromised sensory gating mechanisms. Across pain conditions, EEG transient dynamics showed parietal alpha hyper-synchronization in SCD, particularly elevated centro-parietal alpha power coupled with a localized spatial pattern, indicative of reduced inhibitory control.
CCA network maps revealed extensive slow-wave (δ/θ) activation in SCD, covering approximately 40% of the scalp versus 14% in controls, suggesting compensatory thalamo-cortical recruitment in adults with SCD pain. Focal alpha hubs observed exclusively in controls were absent in SCD, reinforcing disrupted gating hypotheses. This comprehensive analysis provides preliminary evidence, for the first time, revealing a coordinated pattern of cortical dysfunction associated with SCD pain, which may offer new insights to further characterize and better treat SCD pain processing.

Disclosures: **S. Roy:** A. Employment/Salary (full or part-time): Department of Biomedical Engineering, aCarnegie Mellon University. **J. Disu:** A. Employment/Salary (full or part-time): Department of Biomedical Engineering, aCarnegie Mellon University. **N. Mossazghi:** A. Employment/Salary (full or part-time): Department of Biomedical Engineering, aCarnegie Mellon University. **E. Meinert-Spyker:** A. Employment/Salary (full or part-time): Department of Biomedical Engineering, aCarnegie Mellon University. **L. Abdelmohsen:** A. Employment/Salary (full or part-time): Department of Biomedical Engineering, aCarnegie Mellon University. **S. Wood:** A. Employment/Salary (full or part-time): Department of Biomedical Engineering, aCarnegie Mellon University, Department of Electrical and Computer Engineering, Carnegie Mellon University, Neuroscience Institute, Carnegie Mellon University.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.14/N9

Topic: E.01. Somatosensation – Pain and Itch

Title: Convergent Processing of Pain and Visceral Signals by PFC Neurons

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Abstract: The prefrontal cortex (PFC) has been implicated in emotional and autonomic regulation, but its role in integrating visceral and affective signals remains poorly understood. Here, we present the first study combining in vivo calcium imaging of PFC neurons with simultaneous recording cystometric responses (CMG) in freely moving mice to investigate neuronal dynamics during natural bladder voiding and stress-related behaviors. Using miniature microscopy, we recorded PFC cells activity during abdominal pain stimulation, CMG, foot shock, and tail suspension. We found that a substantial subset of PFC neurons were selectively activated by noxious and aversive stimuli (foot shock, tail suspension), consistent with the PFC's role in aversive stress processing. Our findings identify the PFC as a convergence node for processing emotional and visceral inputs, and may provide evidence of its engagement during spontaneous voiding in freely moving animals.

Disclosures: **F. Liu:** A. Employment/Salary (full or part-time): Washington University in Saint Louis. **M. Kawatani:** A. Employment/Salary (full or part-time): Washington University in Saint Louis. **Y. zhang:** A. Employment/Salary (full or part-time): Washington University in Saint Louis. **H. Greenhill:** None. **Z. Lu:** None. **L. Yang:** None. **M. Heitmeier:** None. **V.K. Samineni:** A. Employment/Salary (full or part-time): Washington University in Saint Louis.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.15/N10

Topic: E.01. Somatosensation – Pain and Itch

Support: abc123

Title: Topographical map of subjective states evoked by focal seizures and electrical stimulation of the human insula

Authors: E. MA[1], ***S. PANTIS**[2], W. HUANG[4], V. BUCH[3], J. PARVIZI[4];
[1]Duke Univ., Durham, NC; [2]Stanford Univ., Menlo Park, CA; [3]Neurosurg., Stanford Univ., Palo Alto, CA; [4]Stanford Univ. Sch. of Med., Stanford, CA

Abstract: The posterior insula is recognized as a key region for nociception. However, it remains unclear to what extent distinct neuronal populations in this area are specifically involved in pain, as opposed to also contributing to the processing of temperature or non-noxious, non-thermoceptive somatosensory sensations. This study aimed to address this question by using direct intracranial stimulation of the insula in neurosurgical patients. We recruited 34 patients with focal epilepsy undergoing intracranial EEG monitoring to identify the location of their seizure onset zones (SOZ) and documented the effect of electrical stimulation in discrete insular sites. In total, 278 unique insular electrode pairs were stimulated in either left (42.4%) or right hemisphere (56.8%). Of these, stimulation of 46.4% of sites did not lead to any reportable change in the subject's conscious state (i.e., silent sites). The remaining 53.6% of non-silent sites were more likely to be located in the posterior than anterior insula. Subjective reports elicited by iES were similar between the patients with seizures starting in the insula (N=3) and those with presumably healthy insular cortices (N=31). Of the responsive insular sites, stimulation of only 3.4% (5 sites, 3 patients) induced a clear emotional change (i.e., mostly anxiety) without any other sensations. Stimulation of other responsive sites elicited gustatory/olfactory (1.3%, 2 sites, 2 pts), visceral (10.7%, 16 sites, 9 pts), somatosensory (47.0%, 70 sites, 19 pts), and pain with or without thermoception (35.6%, 53 sites, 11 pts). Among the pain/temperature responses, 36% were purely thermoceptive (e.g., warmth and heat), 25% purely nociceptive (e.g., jabbing pain), 39% were both (e.g., burning pain). The representation of the human body in the posterior insula (in somatosensory, nociceptive and thermoceptive domains) seemed highly scattered and dispersed long the two long gyri but with a clear somatotopic organization: the representation of the lower limbs is largely confined to the posterior long gyrus while scattered neuronal populations across the anterior long gyrus are involved in sensory processing of upper extremities, trunk, head, neck and the throat regions. Our findings are in line with prior reports of subjective states elicited by the stimulation of the human insula, but add novel information about the presence of a somatotopic organization across the two long gyri. Additionally, our findings confirm the presence of scattered and discrete neuronal populations along the posterior insula

that are involved in processing either somatosensory or nociceptive with or without thermoceptive information.

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Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.16/N11

Topic: E.01. Somatosensation – Pain and Itch

Title: Between pain and mind: stress, chronic pain and prefrontal symptoms

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Abstract: Introduction: Pain is an unpleasant sensory experience associated with actual or potential tissue damage. It is classified according to the time of onset as acute pain and chronic pain. Acute pain: This is caused by a physiological response to harmful stimuli, has a sudden onset, and lasts less than 3 months. Chronic pain: It persists or recurs for more than 3 months. Stress is the body's physiological response when faced with a threat. It is acute when it is short-lived; chronic stress can last for weeks or months. When the stressor persists, the HPA axis continues to release corticosteroids, which promotes the release of cytokines. This increases TNF levels, which affects noradrenergic transmission and inhibits the release of norepinephrine, activating its receptors upon prolonged exposure to stress and pain. Prefrontal symptoms are cognitive, emotional, and behavioral disturbances caused by dysfunction in the prefrontal cortex, a key brain region for decision-making, emotional control, and behavioral regulation. Chronic stress and persistent pain can affect prefrontal function, altering the balance between emotion and reason, which can influence personality disorders we observe in daily life. Methodology: An observational, cross-sectional, analytical, and prospective study was conducted to relate stress and chronic pain with prefrontal cortex symptoms in staff at a higher education institution. The perceived stress scale, the McGill-Melzac test to identify chronic pain, and the Prefrontal Symptom Inventory (PSI-20) were used. Descriptive statistics were used, and the Pearson test was used to determine the correlation between variables. Results: Of the 51 participants, 72.5% women and 27.4% men, with a mean age of 39 years, a higher percentage of women reported moderate and high stress (63.6% and 82.3%, respectively), and severe pain was found in 39.2% of participants. Thirty-nine percent of participants had no evidence of altered prefrontal cortex symptoms; however, more women were observed in the moderate and severe symptom category. A statistically significant correlation ($p=0.0008$) was found between chronic pain and prefrontal

cortex symptoms in those with moderate and high stress. Conclusions: A significant association was found between pain and prefrontal symptoms. The more painful the pain, the more difficulties people report with cognitive and emotional management. The results of this study show that, in the presence of chronic pain, there is alteration in prefrontal symptoms, which are responsible for social, emotional, and executive control. Keywords: Stress, Prefrontal symptoms, Chronic pain

Disclosures: I. Pliego-pliego: None. **L. Hernandez Cruz:** None. **A. Osorio-Espinoza:** None.

Poster

PSTR390: Cortical Pain Processing

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR390.17/N12

Topic: E.01. Somatosensation – Pain and Itch

Support: NSTC 112-2410-H-A49-055-M2

Title: The effects of focal muscle vibration combined with exercise on sensorimotor activity in individuals with chronic subacromial impingement syndrome

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Abstract: About 50% of individuals with subacromial impingement syndrome develop chronic symptoms. Chronic subacromial impingement syndrome (CSIS) involves somatosensory and corticomotor alterations. The treatments for these people usually focus on shoulder control and strengthening exercises, but no treatment has been found to target the somatosensory dysfunction. Focal muscle vibration (FMV) provides proprioceptive stimulation and has been shown to improve sensorimotor performance in both healthy and diseased individuals when combined with exercise. This study aimed to investigate the effects of FMV combined with exercise on somatosensory activity, corticomotor excitability, pain, and function in individuals with CSIS. Eight individuals with CSIS were randomly assigned to an FMV or a control group, and received 12 intervention sessions. The FMV group received FMV during 30-minute shoulder exercise training, while the control group received identical training with vibrators turned off. The outcomes were measured before and after the 12-session treatment, including somatosensory activity (using Electroencephalography, EEG), corticomotor excitability (using transcranial magnetic stimulation, TMS), pressure pain threshold (PPT), pain intensity, and shoulder function. Shoulder function was measured with the Disability of the Arm, Shoulder, and Hand scale (DASH) and the patient-specific functional scale (PSFS). Changes in outcomes from baseline to post-test were compared between groups by using independent t-tests. The FMV group showed significant increases in PPT, compared to the control group, in the bilateral upper trapezius and infraspinatus as well as tibialis anterior on the affected side ($p < 0.05$). A significant improvement in PSFS was shown in the FMV group, compared to that in the control

group (p = 0.042). The FMV group showed the trend toward significance in the decreases in sensory-evoked potential amplitude, compared to the control group (p = 0.055). No significant between-group difference was observed in outcomes of corticomotor excitability, pain and DASH. These preliminary findings showed that additional FMV may improve somatosensory processing, reduce central and peripheral sensitization and improve shoulder function in individuals with CSIS.

Disclosures: S. Chiu: None. **Y. Lin:** None. **L. Chou:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.01/N13

Topic: E.01. Somatosensation – Pain and Itch

Support: CONHACYT Grant No. CVU 1291917

Title: Cannabidiol as a potential therapeutic agent for type 2 diabetes induced neuropathic pain in an animal model

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Abstract: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that represents a world-wide health concern. Among its several complications, peripheral neuropathy is one of the most frequently observed in patients with long-standing T2DM. Despite its current treatments, the mechanisms involved in peripheral nerve damage remain unknown. Cannabidiol (CBD) is a phytocannabinoid constituent of Cannabis sativa reported to have neuroprotective and antinociceptive effects. To study the antinociceptive effects of CBD, we induced a neuropathic pain model T2DM and evaluated tactile allodynia following oral administration (p.o.) of three CBD doses (20, 40, and 60 $mg \cdot kg^{-1}$). T2DM and diabetic neuropathy were induced in male and female Wistar rats (n=6) by administering streptozotocin (75 $mg \cdot kg^{-1}$) intraperitoneally on postnatal day five. Neuropathy progression was assessed by measuring mechanical allodynia using the up and down method. Calibrated von Frey filaments (1.4 g-60 g) were used to determine the 50% withdrawal threshold. The animals were divided into five experimental groups: control, diabetic, CB1 antagonist, CB2 antagonist, and serotonin receptor antagonist 5-HT1A (WAY-100635), to evaluate antinociceptive effects and investigate the underlying mechanism of action. Tactile allodynia was reduced during both acute and chronic administration. In acute administration, CBD produced a dose-dependent antinociceptive effect with a maximum possible effect (%MPE) in female (13%, 22% & 31%, respectively) and male

(12%, 23% and 38%, respectively) rats. In both sexes, the administration of WAY-100635 completely reverted the antiallodynic effect of CBD. Both cannabinoid receptor antagonists CB1 and CB2 were administered but failed to revert the effects of CBD. Chronic administration of both 40 $mg \cdot kg^{-1}$ and 60 $mg \cdot kg^{-1}$ of CBD reduced tactile allodynia to values comparable to the control group for an extended period of time. This study suggests that CBD exerts antinociceptive effects in both male and female rats by reducing neuropathic pain caused by T2DM through 5-HT1A receptor activation. No significant differences were observed between sexes in response to both CBD treatments. Furthermore, both acute and chronic administration of both 40 $mg \cdot kg^{-1}$ and 60 $mg \cdot kg^{-1}$ doses produced comparable reductions in tactile allodynia with no significant differences between them. Despite these results, more studies are required to further elucidate the action mechanisms involved. These findings may support the identification of novel therapeutic strategies for managing diabetic neuropathic pain.

Disclosures: D.C. Ledezma: None. **N.L. Caram-Salas:** None. **J. López-Carrillo:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

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Program #/Poster #: PSTR391.02/N14

Topic: E.01. Somatosensation – Pain and Itch

Support: SECIHTI CV 629565

Title: Cannabidiol induces antiallodinic and antihiperalgesic effects via D2-NFKB dopamine receptor in hemiparkinsonian male rats

Authors: *E. GONZÁLEZ MORALES[1], J. SANTAMARIA ANZURES[2], B. GODÍNEZ[3]; [1]UAM-XOCHIMILCO, Mexico City, Mexico; [2]Sistemas Biologicos, [3]Sistemas Biológicos, Univ. Autónoma Metropolitana Unidad Xochimilco, Mexico City, Mexico

Abstract: Neuropathic pain is induced by tissue damage or disease of the sensory nervous system. Most patients with Parkinson's disease have neuropathic pain and do not receive adequate treatment or do not respond to treatment, even though there are various first-line treatments, such as tricyclic antidepressants, gabapentin, pregabalin, duloxetine and fluoxetine. There are alternative treatments such as lidocaine or capsaicin patches, opioids, and cannabis. Cannabis sativa is a plant that contains around 120 compounds. Δ9-THC and cannabidiol (CBD) are the main compounds in the plant. There is currently evidence for the use of CBD in the treatment of anxiety and depressive disorders, psychotic and metabolic disorders such as schizophrenia, diabetes, and pain. One of the neurotransmitter systems that is affected in Parkinson's disease and modulates pain is the dopaminergic system, particularly the dopamine D2 receptor (D2DR), which has been implicated in pain regulation, and one of the possible signaling pathways activated by D2DR is the NFKB pathway. This work aimed to demonstrate whether cannabidiol induce antiallodynic and antihyperalgesic activity in 6-OHDA-lesioned rat

into SNpc. Also, we were interested in investigating whether cannabidiol exerts its antiallodynic and antihyperalgesic effects via D2DR-NFkB-cytokines. For this purpose, we administered increasing doses of CBD (3, 10, 30 and 60 mg/kg, i.p.) in hemiparkinsonian rats with neuropathic pain and evaluated the antiallodynic and antihyperalgesic activity. Subsequently, the D2DR antagonist, L-741, 626 (1.46 and 14.6 nM, i.n.), was administered 10 min before CBD (60mg/kg, i.p.) and evaluated the allodynia and hyperalgesia in 6-OHDA-lesion rats. Finally, we determined the NFkB expression by immunofluorescence and the cytokines production in 6-OHDA-lesion rats with or without CBD treatment (60 mg/kg). Results showed that CBD induces antiallodynic and antihyperalgesic effect and antagonism of D2DR interfered with CBD effects in 6-OHDA-lesion male rats. On the other hand, we find a decrease in NFKB immunoreactivity and cytokines production. These results show that CBD has analgesic activity and exerts it effect by D2DR via NFKB in 6-OHDA-lesion male rats.

Disclosures: E. González Morales: None. **J. Santamaria Anzures:** None. **B. Godínez:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

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Program #/Poster #: PSTR391.03/N15

Topic: E.01. Somatosensation – Pain and Itch

Support: WA State Initiative No. 171

Title: Impact of inflammatory pain on THC-induced sedation, tolerance, and withdrawal in male and female rats

Authors: *C. HICKEY[1], W. GRIMES[1], C. POLING[1], A. R. CALVERT[1], M. M. MORGAN[2]; [2]Dept Psychology, [1]Washington State Univ. Vancouver, Vancouver, WA

Abstract: Delta-9-tetrahydrocannabinol (THC), the primary active ingredient in cannabis, produces a wide range of behavioral effects that include sedation and antinociception. These effects are sensitive to tolerance with repeated administration and tend to be greater in female compared to male rats. Although many people take cannabis to treat pain, little is known about how chronic pain alters the effects of THC. The objective of this experiment was to characterize THC-induced sedation, tolerance, and withdrawal in male and female rats with hindpaw inflammation. Inflammatory pain was induced by injecting CFA into the right hindpaw of male and female Sprague-Dawley rats. Twice daily injections of a relatively low dose of THC (3 mg/kg, s.c.) began on the day following CFA administration and persisted for 7 days. Administration of CFA decreased wheel running to approximately 20% of baseline levels. Wheel running returned to baseline levels within 7 days. Administration of THC caused a consistent decrease in running in female and male rats for all 7 days. Tolerance was assessed on the day following the last THC treatment by injecting all rats with THC. Administration of THC produced a large decrease in wheel running in rats previously treated with vehicle compared to

rats pretreated with THC for 7 days. This tolerance to the sedative effect of THC was more pronounced in male compared to female rats. Dependence was assessed by measuring withdrawal-induced changes in wheel running for 5 days following the THC tolerance test. A small decrease in wheel running occurred 24 - 36 hrs after the last THC injection in female and male rats previously treated with THC. No statistically significant withdrawal-induced changes in wheel running were evident on the remaining days. Although previous research indicates greater antinociceptive effects and greater tolerance in female compared to male rats treated with THC (Wakley et al., 2014), the present data indicate that inflammatory pain reduces or reverses these sex differences. The limited evidence of withdrawal symptoms following termination of THC is consistent with the mild symptoms of spontaneous THC withdrawal reported by others (Aroni et al., 2024) but differ from the enhanced morphine withdrawal in rats with hindpaw inflammation (Kandasamy et al., 2017). In sum, the present data indicate that persistent pain does not alter the sedative effects of THC but does reduce the development of tolerance in female rats and spontaneous withdrawal in both male and female rats.

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Poster

PSTR391: Non-Opioid Pain Treatments

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Program #/Poster #: PSTR391.04/N16

Topic: E.01. Somatosensation – Pain and Itch

Title: Characterization of novel sigma receptor ligands derived from multicomponent reactions as efficacious treatments for neuropathic pain

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Abstract: Neuropathic pain is a significant problem with few effective treatments that lack adverse effects. The sigma receptors (SRs) are known to modulate nociception and have emerged as a potential target for neuropathic pain therapeutics, with sigma receptor antagonists effective analgesics in preclinical models of chronic pain. While sigma-1 receptor (S1R) ligands have been increasingly investigated in this context and are currently in phase I clinical trials, knowledge regarding sigma-2 receptor (S2R) ligands remains limited. To explore this, a novel series of heterocycles through multicomponent reactions. Screening of select compounds in radioligand competition binding assays with fifty CNS targets including G-protein coupled receptors (GPCRs), ion channels, transporters, and enzymes through the NIMH psychoactive drug screening program identified four that display high (nanomolar) binding affinity and

varying selectivity for SR1 and/or SR2 receptors: UVM147, RO-4-3, RO-5-3, and RO-7-3. When screened in vivo at a 30 mg/kg, s.c. dose in CD-1 mice subjected to chronic constriction injury (CCI), RO-5-3 and RO-7-3 displayed significant anti-allodynic effects. Upon full characterization, higher doses (45 and 60 mg/kg) of RO-5-3, but not RO-7-3, significantly attenuated mechanical allodynia in the CCI model of neuropathic pain. In the formalin test of inflammatory pain, both RO-5-3 and RO-7-3 produced dose-dependent antinociceptive effects. To discount potentially confounding effects, effects on locomotor activity, respiration, and reward were also examined in C57BL/6J mice. RO-5-3 induced mild conditioned place aversion and respiratory depression at high doses (45 or 60 mg/kg), without affecting locomotor activity or motor coordination. In contrast, higher doses (30-60 mg/kg) of RO-7-3 caused mild reductions in locomotion, respiratory rate, and motor coordination, but did not induce conditioned place aversion. Collectively, these findings suggest that RO-5-3 exerts antinociceptive and anti-allodynic effects with minimal adverse behavioral effects, supporting the role of SRs in pain modulation. These results add to growing evidence supporting the potential development of SR ligands as efficacious therapeutic agents for neuropathic pain with fewer clinical liabilities.

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Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.05/O1

Topic: E.01. Somatosensation – Pain and Itch

Support: Veterans Affairs

Title: Photoneuromodulation with Green Light Reverses mTBI-Induced Cephalic Pain via GABAergic Circuit Activation in the PAG and DRN

Authors: *J. PARK[1], K. A. ISMAIL[2], V. CURFMAN[2], H. KORAH[2], E. LOUIS[2], C. ANNABI[1], S. WASHINGTON[2], L. MARTIN[1], M. M. IBRAHIM[1];
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Abstract: Photoneuromodulation is emerging as a transformative approach to pain management, offering non-pharmacological strategies with minimal side effects. Among these, green light-emitting diode (GLED) exposure shows promise in preclinical models, yet its mechanisms in brain injury-related pain remain unclear. Here, we investigated the effects of GLED (525-535 nm, 100 lux, 8 h/day) in a weight-drop model of mild traumatic brain injury (mTBI), focusing on cephalic pain and brainstem modulation. mTBI induced persistent periorbital mechanical hypersensitivity, mimicking post-traumatic headache, with no changes in hindpaw sensitivity. GLED exposure both reversed established hypersensitivity and prevented its development when delivered prior to injury. Notably, GLED's antinociceptive effects were

stronger in females, contrasting with previous studies and highlighting potential sex-specific responsiveness. GLED also blunted hypersensitivity reinstated by bright light stimulation (BLS), a trigger of photophobia and headache. Contrary to expectations, GLED did not normalize classical proinflammatory cytokines (TNF-α, IL-1β, IL-6, MCP-1) in the periaqueductal gray (PAG). However, cytokine array revealed a selective reduction of IGFBP-5 and IGFBP-6, suggesting alternate pathways. Western blot showed that GLED reversed mTBI-induced astrocyte activation (GFAP) in the PAG, while microglial marker IBA-1 remained unchanged. Based on prior reports implicating the PAG and dorsal raphe nucleus (DRN) in GLED's effects, we examined neuronal activity in these regions. GLED increased FOS B expression in GABAergic neurons in the PAG and DRN, indicating enhanced inhibition. Concurrently, serotonin levels in the trigeminal nucleus caudalis (TNC) were reduced, possibly reflecting suppressed serotonergic activity from the rostral ventromedial medulla (RVM). We hypothesize that GLED activates GABAergic neurons in the PAG, which in turn inhibit RVM output, reducing descending serotonergic facilitation in the TNC. Together, these findings suggest that GLED alleviates mTBI-induced headache-like pain by engaging inhibitory brainstem circuits and rebalancing descending modulation, positioning photoneuromodulation as a novel, mechanism-based approach to post-traumatic headache, especially in females.

Disclosures: J. Park: None. **K.A. Ismail:** None. **V. Curfman:** None. **H. Korah:** None. **E. Louis:** None. **C. Annabi:** None. **S. Washington:** None. **L. Martin:** None. **M.M. Ibrahim:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.06/O2

Topic: E.01. Somatosensation – Pain and Itch

Support: Department of Defense

Title: New light on recovery: sex differences in post-surgical pain relief with green light photoneuromodulation

Authors: *K. A. ISMAIL[1], J. PARK[2], V. CURFMAN[3], H. KORAH[3], E. LOUIS[3], C. ANNABI[3], S. WASHINGTON[3], L. MARTIN[3], M. M. IBRAHIM[3];
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Abstract: Facing the opioid epidemic, there is a critical need for non-pharmacological pain management strategies, especially for surgical patients prone to anxiety and depression. Green light-emitting diode (GLED) therapy has shown improvement in chronic pain by modulating key pain-processing brain regions. Although the GLED's effects circuitry has been partially mapped, notably from the retina to the PAG and DRN, little is known about how downstream components of the descending pain modulatory pathway, such as the RVM, are engaged. We investigated sex

differences and RVM modulation in a model of postoperative pain. We initiated a comprehensive investigation of GLED effects by mapping whole-brain c-Fos expression to assess modulations of neuronal activity in postoperative rats exposed to white light (WLED) or GLED (525±5 nm, 100lux, 8h/day). Behavioral tests (von Frey, Hargreaves, dynamic weight bearing) were used to measure hypersensitivity and limb functionality following GLED exposure. As GLED modulates the endogenous opioid system in chronic pain, we investigated changes in opioid signaling in the RVM using ELISA and western blot. Additionally, to investigate the connection between the endogenous opioid system and the descending pain pathway, we focused on ON-cell activity, evaluating FosB immunofluorescence in $Tacr1^{+}/Oprm1^{+}$ neurons. Finally, as pain-related neuroinflammation is strongly affected by sex, we investigated the effects of GLED on pro- and anti-inflammatory cytokines in the RVM using ELISA and evaluated glial cell subpopulations using snRNAseq.
Postoperative pain increased c-Fos expression in pain areas such as the somatosensory cortex, the RVM, and the ACC, while GLED significantly reduced cFos expression in these areas. GLED-induced antinociception was more pronounced in males than in females, which correlated with altered expression of the μ-opioid receptor in the RVM. Additionally, female rats exposed to GLED showed increased β-endorphin levels, while males exhibited increased dynorphin levels, illustrating sex-specific differences related to the endogenous opioid system. GLED also significantly reduced FosB expression in RVM $Tacr1^{+}/Oprm1^{+}$ cells, indicating decreased ON-cell activity. Lastly, GLED modulated inflammatory responses in the RVM, rescuing post-surgical elevations in TNFα and restoring IL-10 levels to baseline. GLED mitigates postoperative pain by modulating descending pain pathways, reducing ON-cell activity, and selectively influencing opioid and inflammatory systems in the RVM. Sex-specific differences in GLED's analgesic efficacy show the need for tailored therapeutic approaches.

Disclosures: K.A. Ismail: None. **J. Park:** None. **V. Curfman:** None. **H. Korah:** None. **E. Louis:** None. **C. Annabi:** None. **S. Washington:** None. **L. Martin:** None. **M.M. Ibrahim:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.07/O3

Topic: E.01. Somatosensation – Pain and Itch

Support: NSF HSI-STEM Award #1953448.
TWU Center for Student Research Grant
TWU Research Enhancement Program Grant

Title: Euphorbia bicolor and its potential therapeutic role in regulating pain and neuroplasticity post-burn injury.

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Abstract: Burn injuries are among the most severe forms of trauma, often resulting in persistent pain and long-term sensory dysfunction. Beyond tissue damage, burns induce significant neuroplastic changes in the central and peripheral nervous systems, contributing to the transition from acute to chronic pain. Current treatments, including NSAIDs, opioids, and gabapentinoids, often fail to provide long-term relief and are associated with adverse effects, highlighting the need for novel, non-opioid therapeutics. Our previous studies demonstrated that phytochemicals from the latex extract of *Euphorbia bicolor*, a plant native to the southern U.S., provide long-lasting, non-opioid peripheral analgesia in two rodent models of inflammatory pain. Here, we extend this work in a rat model of full-thickness thermal burn to examine behavioral indicators of affective pain and evaluate changes in sensory afferent input in the dorsal horn of the spinal cord. We hypothesized that *E. bicolor* phytochemicals reduce burn-induced pain chronification and dampen nociceptive afferent input, corresponding to alleviated pain behaviors. Adult male Sprague-Dawley rats (250-350 g) underwent baseline behavior testing followed by full-thickness thermal injury (100°C, 30 s) to the plantar surface of the right hind paw. Reflexive and affective pain behaviors were assessed using the Hargreaves apparatus and mechanical conflict-avoidance system (MACS) at 24 h post-injury. *E. bicolor* extract (500 µg/mL) or vehicle was locally injected at the burn site. Behavioral assessments were repeated at 48 h, 1 wk, 4 wks, and 8 wks post-injection. Rats were then perfused with 4% paraformaldehyde, and lumbar spinal cords (L2-L5) and dorsal root ganglia were collected. Tissue sections (30 µm) were processed for fluorescent immunohistochemistry using antibodies against GAP43, TrkA, and TrkB, markers of axonal sprouting and plasticity. We observed that thermal injury induced robust pain behaviors and increased sensory afferent input to the dorsal horn. These effects were significantly attenuated in *E. bicolor*-treated animals. Our findings suggest that *E. bicolor* phytochemicals exert neuromodulatory effects that disrupt maladaptive plasticity and reduce both behavioral and anatomical correlates of burn-induced pain, supporting its potential as a novel analgesic strategy.

Disclosures: T.P. Olaoluwa: None. **G. Perez Posada:** None. **D.L. Hynds:** None. **C. Maier:** None. **D.L. Averitt:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

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Program #/Poster #: PSTR391.08/O4

Topic: E.01. Somatosensation – Pain and Itch

Support: TWU Research Enhancement Program Grant
TWU Center for Student Research Grant
NSF HSI-STEM Award #1953448

Title: Neural plasticity and affective pain behavior following full-thickness burn injury in rats

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Abstract: Full-thickness burn injuries often result in persistent pain that transitions from acute to chronic states. Current pharmacological interventions, including opioids, NSAIDs, and gabapentinoids, are limited by their side effect profiles and potential for misuse.Understanding the underlying mechanisms of burn-induced pain is critical for developing safer, non-opioid alternatives. Our study investigates affective pain behavior and neural plasticity following thermal injury, using a rodent model to mimic clinically relevant burn conditions. Male and female Sprague-Dawley rats received either a sham treatment or a full-thickness burn to the right hind paw via a 100°C soldering iron applied for 30 seconds. Behavioral assays were conducted at 1-, 4-, and 8-weeks post-injury using the Mechanical Conflict Avoidance System, which evaluates affective and motivational aspects of pain. At 8 weeks, dorsal root ganglia (DRG), lumbar spinal cord segments (L2-L5), and plasma were collected for molecular analyses. We hypothesized that full-thickness thermal injury results in sustained affective pain and remodeling of nociceptive circuitry. Immunohistochemistry using antibodies against GAP43, TrkA, and TrkB was used to assess nerve fiber density and axonal sprouting. Cytokine arrays quantified circulating inflammatory mediators, while Western blotting evaluated changes in GAP43, TrkA, and TrkB expression in neural tissues. Burn-injured rats showed elevated affective pain behavior across the time points compared to sham controls, with evidence of increased axonal branching and altered neurotrophin signaling. These findings suggest chronic changes in pain processing pathways that may underline the transition from acute to chronic pain following thermal injury. Our model reveals key targets associated with neural plasticity and inflammation in chronic burn pain and supports the development of novel non-addictive therapeutics, including plant-derived compounds such as *Euphorbia bicolor*.

Disclosures: G. Perez Posada: None. **T. Olaoluwa:** None. **D.L. Averitt:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.09/O5

Topic: E.01. Somatosensation – Pain and Itch

Title: Antinociceptive effect induced by amatadine in a model of Chronic Constriction Injury of the Rat's Infraorbital

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Abstract: Trigeminal neuralgia (TN) is a disabling sensory disorder with no defined treatment. It is characterized by episodes of persistent pain, either spontaneous or evoked by mechanical stimuli. This condition presents itself as a public health problem because it can cause problems falling asleep, swallowing, and can even affect patients' mood, greatly diminishing their quality of life. Recently, it has been shown that N-methyl-D-aspartate (NMDA) receptors are associated with the development and maintenance of chronic orofacial pain, followed by the direct involvement of reactive oxygen species (ROS). Amantadine is an NMDA receptor antagonist that has demonstrated efficacy in other models of neuropathic pain due to its antioxidant and neuroprotective potential; however, there is insufficient clinical evidence on TN. In this study, the antinociceptive effect (stimulus-dependent hypersensitivity) induced by acute treatment with amantadine (50 mg/kg, i.p.) was evaluated, as well as its ability to modulate oxidative stress by determining lipid peroxidation (LP) and reduced glutathione (GSH) concentrations in the infraorbital nerve and Gasserian ganglion in female Wistar rats with chronic constriction of the infraorbital nerve (CCI-ION). Acute treatment with amantadine decreased nociceptive behaviors, decreased LP, and increased GSH concentrations in injured tissues 21 days post-injury, since nociceptive behaviors are already established. These findings lead us to assume that the development and maintenance of nociceptive behaviors is closely related to oxidative stress and that treatment with amantadine is able to modulate it.

Disclosures: A. Mata-Bermudez: None. **C. Rios:** None. **L. Navarro:** None. **A. Diaz-Ruiz:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.10/O6

Topic: E.01. Somatosensation – Pain and Itch

Title: Niacin as an analgesic against nucleoside reverse transcriptase inhibitor (NRTI)-induced pain

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Abstract: To date, approximately 30.7 million people are receiving combination antiretroviral therapy (cART) to suppress human immunodeficiency virus (HIV) infection. First-line cART includes daily dosing of two separate nucleoside reverse transcriptase inhibitors (NRTIs) as well as a third, variable, antiretroviral. It has been shown that NRTIs induce mitochondrial perturbations resulting in cytosolic leakage of reactive oxygen species (ROS) and the development of NRTI-induced allodynia in up to 60% of people living with HIV (PLWH). Due to the necessity of cART adherence, approximately 37% of PLWH are prescribed long-term opioids to manage NRTI-induced pain. Considering chronic opioid use can lead to tolerance,

addiction, and opioid-induced hyperalgesia, alternative analgesics to alleviate NRTI-induced pain are necessary. To mimic the undetectable and untransmittable viral loads of PLWH who are adherent to cART, we used wild-type (C57BL/6) murine models. Mice were orally dosed for 28 days, via the drinking water, with either the popular NRTI zidovudine (AZT)-only, niacin-only, or AZT+niacin dual treatment. Von Frey and Hargreaves behavior tests were performed as a measure of mechanical and thermal nociception, respectively. To visualize ROS within the spinal cord, a superoxide dye was intrathecally administered 24 hours prior to euthanasia. Upon euthanasia, the lumbar spinal region was sectioned and homogenized to quantify the concentration of nicotinamide adenine dinucleotide (NAD+) - a potent regulator of ROS sequestration. We find that murine models exposed to AZT+niacin dual treatment exhibit significantly increased mechanical and thermal nociceptive thresholds compared to AZT-only groups. Similarly, less visible ROS is observed within the spinal cords of AZT+niacin mice compared to AZT-only groups. Although still downregulated compared to baseline concentrations, concurrent niacin dosing results in increased concentrations of NAD+ within spinal cords. These results suggest that niacin administration upregulates NAD+ concentrations within the spinal cord, reduces the presence of ROS, and partially alleviates both mechanical and thermal allodynia brought about by NRTI administration. We propose that NAD+-mediated sequestration of ROS, as well as other antioxidant therapies, may be plausible alternatives to opioids when treating NRTI-induced pain.

Disclosures: M. Maurelli: None. **S. Tang:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

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Program #/Poster #: PSTR391.11/O7

Topic: E.01. Somatosensation – Pain and Itch

Support: RS-2023-00209591
RS-2023-00243616

Title: Injectable dual-drug hydrogel system for sustained drug delivery to treatneuropathic pain

Authors: *D. LEE[1], S. KIM[2], M. KIM[2], G.-B. JU[2], S. SOHN[3];
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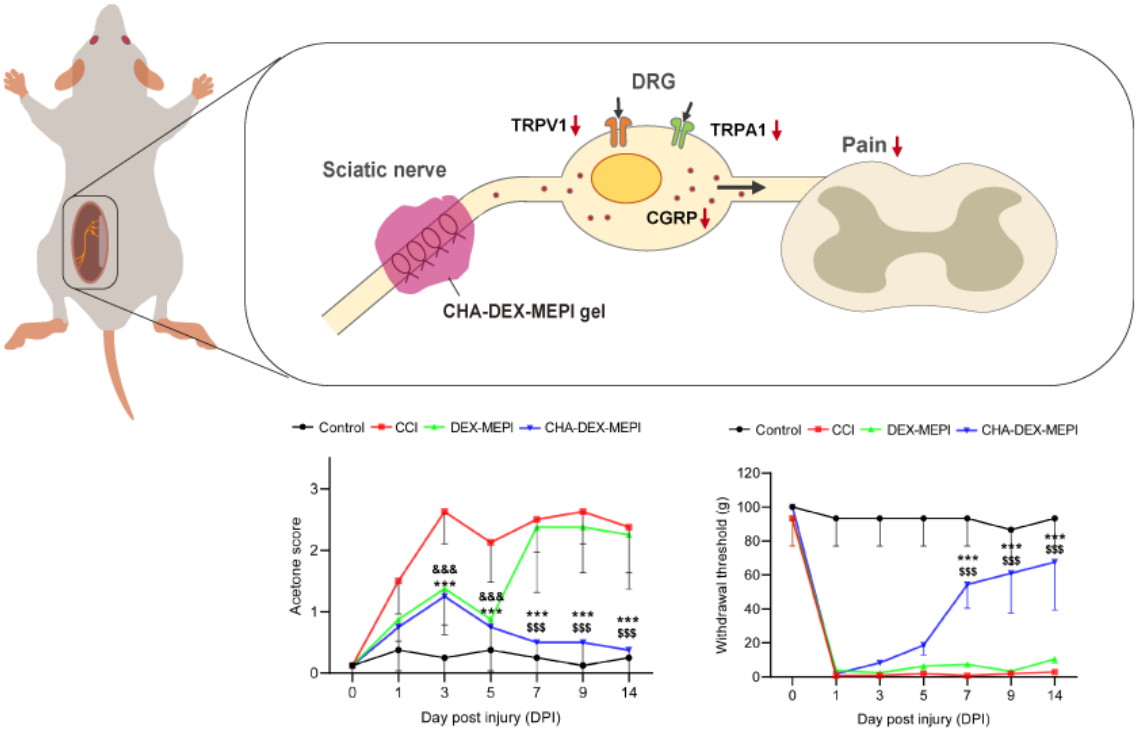
Abstract: Spinal epidural injections remain a clinical challenge due to their limitations, including short drug durations and accidental drug flows to motor nerves. In this study, we developed and evaluated a CHA-DEX-MEPI hydrogel as a novel injectable drug delivery system to provide sustained pain relief and reduce temporary flow to motor nerves. Glycolic chitosan (gC) and oxidized hyaluronic acid (oHA) were used to form what is referred to as the gC/oHA

(CHA) gel through self-crosslinking. Dexamethasone (DEX) and mepivacaine (MEPI) were embedded with the CHA gel to synthesize the CHA-DEX-MEPI gel. Bone marrow-derived macrophages (BMMs) were activated by lipopolysaccharide (LPS) to evaluate the anti-inflammatory effect for three days. The chronic constriction injury (CCI) model was employed in Sprague-Dawley (SD) rats to investigate the pain-relief effects for 14 days. The structure of the CHA gel indicated injectable viscosity and porosity. The CHA-DEX-MEPI gel sustainably released DEX and MEPI for more than 72 hours. It prolonged the reduction of inflammatory cytokines genes for three days in the BMMs compared to that in a DEX-MEPI solution. Moreover, our results demonstrated that the CHA-DEX-MEPI gel significantly reduced both cold and mechanical allodynia after seven days post-injury (DPI). It also demonstrated sustained reductions in the expression levels of key nociceptive markers, including transient receptor potential vanilloid type-1 channel (TRPV1), transient receptor potential ankyrin 1 (TRPA1), and calcitonin-gene-related peptide (CGRP), on 14 DPI. These findings suggest that the CHA-DEX-MEPI gel can serve as a promising approach for the treatment of neuropathic pain.



Disclosures: D. Lee: None. **S. Kim:** None. **M. Kim:** None. **G. Ju:** None. **S. Sohn:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

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Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant 1R01DK134893-01
TTUHSC Start-up Fund

Title: Inhibition of Integrated Stress Response Alleviates Pain in Diabetic Neuropathy

Authors: A. MISRA[1], V. S. THAKUR[2], C. VALDEZ[2], I. PRADHAN[3], B. MORALES[4], M. YOUSUF[5], *M. CHATTOPADHYAY[6];
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Abstract: Introduction: Diabetic painful neuropathy (DPN) is one of the most debilitating complications of diabetes that affects almost one third of diabetic patients and interferes with their quality of life. Recent evidence suggests that increases in the plasma levels of methylglyoxal (MGO) in diabetes induces the integrated stress response (ISR) in nociceptors which leads to development and progression of DPN. This study demonstrates how ISR in diabetic condition facilitates the development of DPN and how inhibition of ISR using ISRIB could alleviate DPN. **Materials and Methods:** Male Zucker diabetic fatty (ZDF) and lean rats were used. One set of male ZDF rats were treated with ISRIB (2.5 mg/kg, IP) twice a week for 6 weeks starting at the onset of diabetes. We assessed evoked pain (von Frey filaments, Hargreaves, and acetone tests) in the lean, diabetic and ISRIB treated ZDF animals. The electrophysiology studies were assessed for sensory amplitude and nerve conduction velocity. Blood was collected biweekly to quantify levels of MGO in the plasma using ELISAs. Animals were euthanized after the treatment regimen and spinal cord, DRG, sciatic nerve as well as footpad were harvested to compare the expression of stress-resistant genes like Xbp1, Atf3, and Ddit3 as well as damage-associated markers such as ninjurin-1, neurofilament-L (NF-L), GAP43 in the DRG, spinal cord and sciatic nerves between the diabetic vs ISRIB-treated groups along with the respective lean-control animals. **Results:** ZDF rats are spontaneously diabetic at 10-12 weeks of age and exhibited thermal hyperalgesia, mechanical as well as cold allodynia compared lean controls at 14 weeks of age. Diabetic ISRIB treated animals showed alleviation of pain hypersensitivity as observed from the thermal, mechanical as well as cold pain tests. Diabetic animals demonstrated increased ninjurin-1, iNOS, activating transcription factor (ATF)-3 expression and decreased expression of growth associated protein (GAP)-43 in the DRG and spinal cord compared to control and treatment groups. ZDF animals with ISRIB treatment also showed differential expression of a number of damage-associated markers such as NF-L, GAP43 in the DRG, spinal cord as well as in the sciatic nerve compared to ZDF animals. **Conclusion:** This study demonstrates that treatment with small molecule inhibitor of integrated stress response, ISRIB may alleviate the damage associated markers as well as stress-resistant genes and proinflammatory mediators that are differentially expressed in the ZDF animals with PDN compared to the lean counterparts which may help optimize treatment options for this debilitating condition.

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Poster

PSTR391: Non-Opioid Pain Treatments

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Program #/Poster #: PSTR391.14/O9

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant R18EB035005

Title: Diagnostic Ultrasound-Guided Focused Ultrasound-Induced Non-Invasive, Reversible Peripheral Nerve Inhibition in an In Vivo Model of Acute Pain: A Proof-of-Concept Study

Authors: *N. SANGWAN[1], T. ANDERSON[2];
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Abstract: Moderate to severe acute pain after surgery is common and associated with worsened patient outcomes; current pain management therapies, including traditional local anesthetic- and needle-based peripheral nerve blocks (PNBs), have shortcomings limiting their use. We investigated the efficacy of diagnostic ultrasound (dUS)-guided focused ultrasound (FUS)-induced PNB to non-invasively, reversibly inhibit the sciatic nerve in a rodent model of acute, incisional pain. Adult male and female Sprague-Dawley rats (200-400g) were randomly assigned to an Intervention Arm (INT: FUS application + hindpaw (HP) incision) or a Control Arm (HP incision only). After inducing general anesthesia and depilation, animals were placed in a heated water bath. Co-aligned dUS and FUS transducers were utilized to visualize and apply energy non-invasively (transcutaneously) to the sciatic nerve. FUS parameters were iteratively adjusted until a parameter set was determined, resulting in reversible nerve inhibition (6.65 megapascals, 100% duty cycle, 50 millisecond burst period, total application time 60 seconds; ISPPA, spatial peak temporal average intensity = 1436 watts cm-2). After FUS application, a HP incision was performed. Behavioral testing was conducted before and after FUS application/HP incision for up to 24 weeks. The primary outcomes were changes over time in HP thermal withdrawal latency (modified Hargreaves) and mechanical withdrawal threshold (Randall-Selitto) in INT compared to Control. Secondary outcomes were changes over time in HP extension and flexion in INT animals compared to Control. Compared to Control animals (N=6), INT animals (N=11) had: 1) increased thermal nociceptive thresholds for 4 weeks; 2) increased mechanical nociceptive thresholds for 4 weeks; 3) decreased HP flexion for 5 weeks; and 4) decreased HP extension for 7 weeks (Figure 1a-d). In a rat model of acute incisional pain, using co-aligned dUS and FUS transducers to non-invasively apply ultrasound energy to the sciatic nerve, we determined a set of FUS parameters that resulted in reversible inhibition of sensory and motor

behaviors. Future investigations will: 1) Seek to determine FUS parameters that result in shorter and/or variable duration reversible, nociceptive fiber inhibition with minimal to no motor and non-pain sensory fiber inhibition, and 2) Use microscopy to evaluate changes over time to the peripheral nerve structure after FUS application. The findings from this proof-of-concept study suggest that dUS-guided FUS-induced PNB holds promise as a novel therapeutic tool to non-invasively, reversibly inhibit peripheral nerves to prevent and treat pain.

Disclosures: N. Sangwan: None. **T. Anderson:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

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Topic: E.01. Somatosensation – Pain and Itch

Support: DHA grant R.0036257.4.4.1

Title: Long-lasting analgesic effects of (2R,6R)-Hydroxynorketamine in a chemotherapy-induced peripheral neuropathy mouse model.

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Abstract: Background: Chemotherapeutic agents, such as cisplatin and oxaliplatin, are commonly used to treat different types of cancer. However, these drugs often induce a severe form of neuropathy known as chemotherapy-induced peripheral neuropathy (CIPN) that can limit cancer treatments. There are no approved drugs for treating the neuropathy, with duloxetine providing limited benefits. Opioids may be used, however, their use is limited by tolerance and risk of abuse. New long-lasting non-opioid analgesics are urgently needed to treat CIPN. In the last few years, (2R,6R)-Hydroxynorketamine ((2R,6R)-HNK) has been demonstrated to exert potent long-lasting analgesic effects in mouse models of acute and chronic pain. Our goal in the present study was to investigate the potential analgesic effects of (2R,6R)-HNK using a CIPN model induced by oxaliplatin (OXA) in mice. Methods: C57BL/6J male mice received OXA (3 mg/kg, i.p.) treatment for 2 cycles of 5 days to induce CIPN. One week after induction, they were tested for mechanical sensitivity using the Von Frey test. Animals then received one injection of (2R,6R)-HNK 30 mg/kg i.p., and were tested 24 h after. Two weeks later, they received repeated treatment with (2R,6R)-HNK for 7 days and were then tested until no drug effects were observed. In a separate experiment with the goal of elucidating the pharmacological mechanisms involved, different drugs were injected (i.p.) 30 min before (2R,6R)-HNK and animals tested 24 h later: 1) NBQX (AMPA receptor antagonist); 2) naltrexone (opioid antagonist); 3) VU6001966 (mGLUR2 Negative Allosteric Modulator); and 4) LY341495

(mGLUR2/3 antagonist). Results: OXA induced a pronounced mechanical hypersensitivity whereas treatment with (2R,6R)-HNK was able to reverse it 24 h after a single injection. Repeated treatment with (2R,6R)-HNK persistently counteracted the mechanical hypersensitivity with animals presenting higher paw withdrawal thresholds for 7 days. After treatment cessation, paw withdrawal thresholds remained elevated for up to 21 days. Treatments with NBQX, VU6001966 and LY341495 blocked (2R,6R)-HNK-induced analgesia, suggesting the involvement of glutamatergic mechanisms in the analgesic response. In contrast, naltrexone had no effects on mechanical sensitivity. Conclusions: (2R,6R)-HNK produced long-lasting analgesic effects in a CIPN mouse model without tolerance that persisted following treatment cessation. Pharmacological experiments point to the involvement of AMPA, as well as mGLUR2 and mGLUR2/3 receptors in (2R,6R)-HNK-induced analgesia. Opioid receptors appear to have no involvement, since naltrexone failed to block these effects.

Disclosures: B. Carabelli: None. **C. Danzy:** None. **C.A. Browne:** None. **I. Lucki:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.16/O11

Topic: E.01. Somatosensation – Pain and Itch

Support: SECITHY 956067

Title: Rotigotine decreases allodynia and hyperalgesia by activation of dopamine D2/D3 receptors in hemiparkinsonian male rats

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder with motor symptoms such as bradykinesia, rigidity, and tremors. PD is caused by a loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in a dopamine deficit. Also, patients with PD present several non-motor symptoms, such as depression, sleep disturbance, fatigue, and pain. Pain is one of the most common non-motor symptoms of PD, impacting the quality of life of the patient negatively. The present study aims to investigate the possible anti-allodynic and anti-hyperalgesic effect of rotigotine in 6-OHDA-lesioned rats and determinate if the rotigotine effect is through the activation of D1, D2, and D3 dopaminergic receptors and the consequence reduction in proinflammatory cytokines production (IL-1β, IL-6, and TNFα). The anti-allodynic and antihyperalgesic effect of rotigotine (0.5mg/kg, i.n.) was evaluated in acute (8 hours) and subacute (5 days) administration in 6-OHDA-lesioned rats. Intranasal administration

of rotigotine reverted the acute and subacute allodynia and hyperalgesia in 6-OHDA-lesioned male rats. In addition, co-administration of nafadotride (3 nmol, i.n.) a selective D3 receptor antagonist plus rotigotine blocked the antiallodynic and antihyperalgesic effects induced by rotigotine. Likewise, coadministration of L-741,626 (14.6 nmol, i.n.) a selective D2 receptor antagonist plus rotigotine decreases the antinociceptive effect of rotigotine. Finally, co-administration of a D1 receptor antagonist SCH 23390 (3 nmol, i.n.), plus rotigotine did not decrease the antinociceptive effects induced by rotigotine. The different dopaminergic antagonists alone did not present an effect on the allodynia and hyperalgesia in 6-OHDA-lesioned rats. These results indicate the involvement of dopamine D3/D2 but not D1 receptors in pain modulation. Additionally, we found a decrease in proinflammatory cytokines production in rats with rotigotine treatment. In conclusion, the antinociceptive effects of rotigotine occur via D2/D3 but not D1 dopamine receptor and the final signaling pathway activated by D2/D3 dopamine receptors, probably by NFKB, inhibit proinflammatory cytokine production in 6-OHDA-lesioned rats and is related to the development and maintenance of pain.

Disclosures: J. Santamaria: None. **E. González Morales:** None. **J. Santamaria Anzures:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DFFEEWREFE. **B. Godínez:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.17/O12

Topic: E.01. Somatosensation – Pain and Itch

Title: Salvianolic Acid B Alleviates Osteoarthritis-Associated Pain in a Rat Model

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Abstract: Osteoarthritis (OA) is a common and debilitating degenerative joint disease characterized by the progressive breakdown of articular cartilage, chronic inflammation, and pain, particularly in weight-bearing joints. Despite its high prevalence and impact on quality of life, current therapeutic strategies primarily focus on symptom management and offer limited efficacy in halting or reversing disease progression. There is a growing interest in identifying novel agents that can address both the symptomatic and structural aspects of OA. Salvianolic acid B (SAL-B), a natural phenolic compound extracted from *Salvia miltiorrhiza*, has demonstrated potent antioxidant and anti-inflammatory properties, making it a promising candidate for OA treatment.This study aimed to evaluate the therapeutic potential of SAL-B in an OA in vivo rat model. OA was experimentally induced in rats, after which SAL-B was administered. To assess the therapeutic effects, a series of behavioral and functional tests, including rotarod performance, dynamic weight-bearing analysis, von Frey filament testing, and

hotplate response, were conducted to evaluate motor coordination, nociceptive sensitivity, and overall locomotor function.OA was induced in rats, followed by administration of SAL-B. Behavioral and functional assessments, including rotarod performance, dynamic weight-bearing, von Frey filament testing, and hotplate response, were conducted to evaluate motor coordination, pain sensitivity, and overall mobility. Inflammatory markers, specifically TNF-α and IL-6, were quantified to assess systemic inflammation. Additionally, micro-computed tomography (micro-CT) was employed to analyze subchondral bone architecture, focusing on bone volume and trabecular integrity.The results demonstrated that SAL-B treatment significantly improved motor function and mobility, reduced mechanical and thermal allodynia, and decreased levels of pro-inflammatory cytokines. Furthermore, micro-CT analysis revealed preservation of subchondral bone structure in SAL-B-treated rats compared to controls. These findings suggest that SAL-B not only alleviates OA-associated pain but also contributes to the preservation of joint integrity.In conclusion, SAL-B exhibits significant therapeutic potential in the management of OA by targeting both inflammatory and structural components of the disease. Further studies are warranted to explore its mechanisms of action and potential clinical applications.

Disclosures: A. Khan: None. **H. Sabella:** None. **F. Deba:** None. **A. Rivera:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.18/O13

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant 5R01NS133572
NIH Grant 5T32DA043469

Title: Pain modulation by M2 muscarinic acetylcholine receptors in the ventrolateral periaqueductal gray

Authors: *Y. YIN, D. S. MCGEHEE;
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Abstract: Chronic pain affects over 1/5th of adults in the United States, and 22.1% of those individuals have used a prescription opioid in the past 3 months. While opioids are effective for pain relief, they carry serious adverse side effects including dependence and tolerance. These challenges underscore the need to identify novel pain control mechanisms and develop safer, non-opioid pain therapies. A critical analgesic target of opioids is the descending pain pathway, particularly the ventrolateral periaqueductal gray (vlPAG). Another important neuromodulator that has analgesic effects within this circuitry is acetylcholine (ACh). Our previous work demonstrated that pain conditions suppress ACh levels in the vlPAG in mice and that stimulation of cholinergic input to vlPAG is analgesic. The goal of this work is to investigate how ACh receptors in this nucleus modulate pain signaling. Using fluorescent in situ hybridization, we

found strong expression of M2 muscarinic acetylcholine receptors (mAChRs) on GABAergic neurons in the vlPAG. To assess the functional activity, we expressed the calcium indicator, GCaMP8s, in GABAergic neurons in the vlPAG using a Gad2-Cre mouse line. To model chronic inflammatory pain, we injected Complete Freund's Adjuvant (CFA) into the hind paw of the mice. Both saline- and CFA-treated mice then underwent fiber photometry recordings during a radiant heat source (RHS) behavioral assay to simultaneously monitor vlPAG GABAergic neuronal activity and pain-like behaviors. Three days after CFA injection, mice exhibited reduced paw withdrawal latency to RHS, indicative of thermal hyperalgesia. Concurrently, the mean fluorescence change of calcium signal from vlPAG GABAergic neurons was elevated relative to baseline, suggesting increased GABAergic neuronal activity. Systemic administration of an M2 mAChRs agonist in these CFA-treated mice increased their paw withdrawal latency and reduced vlPAG GABAergic activity close to baseline levels. Conversely, systemic injection of an M2 mAChR antagonist in saline-treated mice reduced paw withdrawal latency and increased vlPAG GABAergic neuronal activity. Together, our data suggest that pain-induced suppression of ACh transmission reduces M2 mAChR activity, leading to disinhibition of vlPAG GABAergic neurons to lower pain thresholds. This work provides new insight into cholinergic modulation in the descending pain pathway and identifies M2 mAChRs as a potential target for non-opioid pain treatment.

Disclosures: Y. Yin: None. **D.S. McGehee:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.19/O14

Topic: E.01. Somatosensation – Pain and Itch

Support: DA055578

Title: Inhibition of N-acylethanolamine Acid Amidase (NAAA) Produces Sustained Analgesia and Suppresses Development of Opioid Tolerance

Authors: *L. BOULLON[1], L. ASSIS[1], R. BARKER[1], D. PIOMELLI[2], A. G. HOHMANN[1]; [1]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Univ. California Irvine, Irvine, CA

Abstract: Introduction: The enzyme N-acylethanolamine acid amidase (NAAA) degrades the anti-inflammatory lipid palmitoylethanolamide (PEA), a process implicated in pain processing and chronification. We evaluated the analgesic efficacy, safety, and mechanisms of action of the NAAA inhibitor ARN19702 in mouse models of inflammatory and neuropathic pain. We also assessed its potential to enhance opioid therapy by mitigating morphine-induced tolerance and side effects. **Methods:** Inflammatory pain was induced by intraplantar injection of complete Freund's Adjuvant (CFA), and neuropathic pain by unilateral spared nerve injury (SNI).

Mechanical hypersensitivity was measured before and after injury and following acute or chronic pharmacological treatments. ARN19702 was administered intraperitoneally (i.p.) at varying doses to assess antinociceptive efficacy and duration of action. Chronic treatment with ARN19702 (10 mg/kg) was evaluated over 12 days in the SNI model. To investigate opioid-sparing effects, mice received repeated doses of vehicle, morphine (10 mg/kg), ARN19702 (1 mg/kg), or their combination. Mechanistic studies included the use of conditional knockout mice lacking NAAA in CD11b-expressing cells to evaluate microglia/macrophage involvement. **Results:** ARN19702 dose-dependently reversed CFA- and SNI-induced mechanical hypersensitivity, with a minimum effective dose of 10 mg/kg and effects lasting up to 24h. Chronic ARN19702 treatment maintained antinociceptive efficacy without tolerance, unlike morphine. A subtherapeutic ARN19702 dose attenuated morphine tolerance but exacerbated withdrawal symptoms after cessation of morphine treatment. The antinociceptive effects of ARN19702 were absent in $CD11b^{CRE/+}NAAA^{fl/fl}$ mice, implicating involvement of myeloid-lineage microglia/macrophages. **Conclusions:** NAAA inhibition with ARN19702 provides robust, sustained analgesia in inflammatory and neuropathic pain. It avoids opioid-like tolerance and may modulate microglial/macrophage activity to exert its effects, representing a promising strategy for safe and effective pain management.

Disclosures: L. Boullon: None. **L. Assis:** None. **R. Barker:** None. **D. Piomelli:** None. **A.G. Hohmann:** None.

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.20/O15

Topic: E.01. Somatosensation – Pain and Itch

Title: Evaluating the in vivo efficacy of NLRP3 inhibitor, NT-0249, in pre-clinical models of chronic joint pain

Authors: *P. THORNTON, N. CLARKE, Z. DIGBY, V. READER, N. LINDSAY, D. HARRISON, A. BILLINTON, A. P. WATT;
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Abstract: Current therapies for chronic pain lack efficacy and this debilitating condition now affects over 25% of the US population. Inflammasomes, critical components of the immune system, control cytokine release, which in turn modulates sensory neuron function. In addition, their regulation of microglial IL-1beta release may facilitate central sensitisation and drive enhanced nociceptive signaling. The NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome in particular, has been implicated in the initiation and maintenance of chronic pain states and may represent a novel, druggable target for the treatment of pain. NT-0249, a potent, selective, brain-penetrant NLRP3 inhibitor, currently in clinical development has demonstrated peripheral and central anti-inflammatory effects in diverse pre-clinical models,

including peritonitis, cryopyrin-associated periodic syndrome (CAPS) and diet-induced obesity[1,2]. To evaluate the therapeutic potential of NT-0249 in pain, we employed a multi-step approach involving in vitro, pharmacokinetic, and in vivo efficacy studies. Human iPSC-derived microglial cultures were used to assess NT-0249 potency by measuring its effects on NLRP3-dependent IL-1beta release. CSF/brain exposures were assessed in rats using intracerebral microdialysis. NT-0249 was subsequently tested in mouse models of Complete Freund's adjuvant (CFA) or monoiodoacetate (MIA)-induced mechanical hypersensitivity. In human iPSC-derived microglial cultures, NT-0249 blocked NLRP3-dependent IL-1beta release with a potency of 6.3±1.3 nM (mean±SD). During rat microdialysis, NT-0249 achieved robust CSF exposure (Kp,uu/CSF=0.62) following oral dosing (30 mg/kg). In dose-response studies in mice, 10, 30 or 100 mg/kg of NT-0249 fully reversed CFA-driven mechanical hypersensitivity with significant target coverage (>50%) sustained for >6 h. In the MIA model, 100 mg/kg NT-0249 reversed mechanical hypersensitivity in both early (Days 3-7), and late (Days 24-28) disease phases. Collectively, these data demonstrate that NT-0249 potently inhibits NLRP3 in microglia, accesses the CNS following oral dosing, and reverses mechanical hypersensitivity in two translational models of pain. These data provide additional support for a role of NLRP3-dependent mechanism(s) in the maintenance of mechanical hypersensitivity. Furthermore, our clinical-stage candidate, NT-0249, is well positioned to target clinical pain states via inhibition of ongoing NLRP3 activity, and further studies are warranted to explore this.[1]J Pharmacol Exp Ther 2024 Feb 15;388(3):813-826; [2]ACS Pharmacol Transl Sci 2024 Apr 18;7(5):1438-1456.

Disclosures: P. Thornton: A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. **N. Clarke:** A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. **Z. Digby:** A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. **V. Reader:** A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. Other; VR has patents on NT-0249. **N. Lindsay:** A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. **D. Harrison:** A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. Other; DH has patents on NT-0249. **A. Billinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. **A.P. Watt:** A. Employment/Salary (full or part-time): NodThera. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NodThera. Other; APW is Chair of Babraham Institute Enterprise Board, Cambridge, UK. APW has patents on NT-0249..

Poster

PSTR391: Non-Opioid Pain Treatments

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR391.21/O16

Topic: E.01. Somatosensation – Pain and Itch

Support: NIH Grant RM1DE033491
NIH Grant NS102722
NIH Grant DE029951
DoD Grant W81XWH1810431
DoD Grant W81XWH2210239

Title: Inhibition of Neuropilin-1 and Nerve Growth Factor interaction as a non-opioid strategy for pain modulation

Authors: *E. DAMO[1,2], R. BRUNI[1,2], R. TONELLO[1,2], M. PANJALINGAM[3], S. SAURABH[3], N. W. BUNNETT[1,2];
[1]Mol. Pathobiology, [2]Pain Res. Ctr., NYU Dent., New York, NY; [3]Chem., New York Univ., New York, NY

Abstract: Chronic pain affects 20% of the global population and treatments are often ineffective or cause severe side effects, underscoring the need for safer, non-opioid therapies. Nerve Growth Factor (NGF) is a validated non-opioid target for pain management, but systemic NGF blockade via monoclonal antibodies has been linked to joint damage, limiting its clinical utility. We identified Neuropilin-1 (NRP1), a non-signaling transmembrane receptor, as a novel co-receptor and modulator of NGF-induced pain signaling and sought to determine the molecular mechanics of the NRP1-NGF interaction and its therapeutic potential. To explore this interaction, we used crosslinking assays and peptide arrays. Crosslinking confirmed direct NGF-NRP1 binding via the b1 domain's arginine pocket, which was blocked by the b1-binding peptide Tuftsin. Peptide arrays localized the NRP1 binding site to the NGF C-terminus at the C-end rule (CendR) motif. We used super-resolution single-molecule localization microscopy to visualize NGF-induced redistribution of NRP1 at the plasma membrane. NGF treatment triggered faster diffusion of NRP1 single molecules and transient peripheral clustering of NRP1 in HEK293T cells, consistent with ligand-induced signalosome formation. To assess functional relevance, we tested NRP1-targeting agents in the sodium monoiodoacetate (MIA)-induced osteoarthritis (OA) model in mice. Mice received an intra-articular (i.a.) injection of MIA or saline via the infra-patellar ligament of the left knee. NRP1 inhibitors were administered (i.a.) on days 10 and 14 post-MIA. Mechanical sensitivity (von Frey, d10), weight-bearing distribution (weight-bearing apparatus, d14) and motor coordination (rotarod test, d14) were tested. A NRP1-blocking monoclonal antibody (Vesencumab, i.a.), a CendR peptide (i.a.), and intrathecal NRP1 shRNA each reduced mechanical allodynia, improved weight-bearing symmetry, and enhanced motor function in male mice. Effects in females were more modest but present. None of the agents affected sham-treated animals. Together, these findings identify NRP1 as a key modulator of NGF-driven pain signaling. By selectively disrupting NGF-NRP1 interactions, NRP1-targeting strategies effectively attenuate pain-related behaviors without the adverse effects associated with global NGF inhibition. Inhibition of NRP1 represents a promising, safer, non-opioid therapeutic approach for the treatment of chronic pain.

Disclosures: E. Damo: None. **R. Bruni:** None. **R. Tonello:** None. **M. Panjalingam:** None. **S. Saurabh:** None. **N.W. Bunnett:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Takeda Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Endosome Therapeutics.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.01/P1

Topic: E.03. The Chemical Senses

Support: Tokai Pathways to Global Excellence (T-GEx), part of MEXT Strategic Professional Development Program for Young Researchers

Title: The distribution of taste buds in the oral cavity and activation of brain regions including gustatory neurons by feed in Japanese eel larvae

Authors: *H. HAGIO[1,2], H. YOKOYAMA[2], Y. YAMADA[3], N. YAMAMOTO[2];
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Abstract: Many researchers have been engaged in the establishment of full life-cycle aquaculture of Japanese eel (*Anguilla japonica*), and some have succeeded in it. However, we must solve some problems for commercialization. One of them is low growth rate of eel larvae under aquaculture, and we need to improve feeds for eel larvae. We studied activation of the brain by feed to understand important brain regions to enhance feeding behavior. We also examined distribution of taste buds, which play an important role in feeding behavior, in the oral cavity of eel larvae to understand development of gustatory sensory organ with growth.
We performed immunohistochemistry for markers of taste buds and for α-Tubulin on whole heads and transverse head sections to examine the distribution of taste buds and nerves in the oral cavity of eel larvae. Our results showed that the number of taste buds increased with growth. We made Nissl-stained brain sections of eel to identify brain regions. Then, we fed eel larvae and conducted immunohistochemistry for markers of neuronal activation. A lot of brain regions including gustatory and other sensory neurons were activated by feed. Our results suggest that the eel larvae have more taste buds as they become older and gustatory neurons are activated by feed. We think that gustatory sense is important for eel larvae to enhance feeding behavior. We need to examine other brain regions and other sensory systems activated by feed in eel larvae.

Disclosures: H. Hagio: None. **H. Yokoyama:** None. **Y. Yamada:** None. **N. Yamamoto:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.02/P2

Topic: E.03. The Chemical Senses

Support: Georgia State University Neuroscience Institute Funding (no external support)

Title: The cystic fibrosis transmembrane conductance regulator (CFTR) channel modulates the functional output of human fungiform taste bude cells (TBCs)

Authors: *S. IYER, J.-P. MONTMAYEUR, C. DOTSON;
Georgia State Univ. Neurosci. Inst., Atlanta, GA

Abstract: Cystic fibrosis (CF) is a recessive genetic disease primarily characterized by severe respiratory dysfunction. It is caused by genetic mutations [CSD1] in CFTR, an ion channel permeable to chloride and bicarbonate and regulated by phosphorylation and binding of ATP. People with CF experience dysregulated feeding and metabolic deficits that are often attributed to CFTR-related pancreatic and gastrointestinal dysfunction. However, CFTR has also been found in TBCs, and although its function remains unknown, studies have shown that CFTR can regulate immune responses of nasal epithelial cells which, interestingly, are mediated by bitter taste receptors expressed in these cells. Given the importance of taste in food selection and consumption, we postulated that CFTR may impact peripheral taste signaling at the level of taste receptor-expressing TBCs (the primary sensory organs of the taste system), which may contribute to the CF-related nutritional and metabolic deficits referenced above. To begin to test this hypothesis, we assessed how pharmacological inhibition of CFTR could affect functional responses of cultured human fungiform taste bud cells to prototypical taste stimuli (e.g., bitter, sweet, fat) using single cell calcium imaging and neurotransmitter (ATP) release measurements. We first established the presence of CFTR in these cells using immunocytochemistry and RT-PCR. We next found that CFTR inhibition generally increased stimulus-evoked calcium responses but that the specific response parameters impacted varied across different stimuli, which we postulate is due to differences in signal transduction mechanisms and the involvement of store-operated calcium channels. For example, response amplitudes to bitter and sweet stimuli were significantly enhanced with no changes in the proportion of cells responding to these stimuli whereas the opposite trends were observed with a fatty acid stimulus. Additionally, bitter-evoked ATP release was significantly enhanced by CFTR inhibition, suggesting that these effects are reflected throughout the signal transduction cascade. Ongoing and future experiments utilize siRNA knockdown as well as intra- and extracellular electrophysiology to further explore the impacts of CFTR. In addition to human TBCs, we have also detected CFTR in mouse taste tissues and mined mouse TBC RNA sequencing datasets to determine CFTR co-expression patterns and guide future cellular and behavioral experiments in mice. Taken together, these data suggest that CFTR can modulate the signaling output of the taste bud and thus is a potential therapeutic target for feeding and metabolic health, especially in the context of CF.

Disclosures: S. Iyer: None. **J. Montmayeur:** None. **C. Dotson:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.03/P3

Topic: E.03. The Chemical Senses

Support: RO1 1151776-1-83715

Title: Caffeine consumption drives changes in salivary protein expression.

Authors: *E. DEMIERI;
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Abstract: Bitter taste often acts as a sign of a potential toxin and are often aversive. However, many bitter foods are consumed in our everyday lives. Our lab has been focused on the role of salivary proteins in altering bitter taste. There are several examples in the human literature of caffeine acceptance being linked to specific salivary proteins, but the relationship is correlational, are the salivary proteins increased by high exposure to caffeine or do they alter the taste of caffeine altering intake? Here we use a rodent model to ask if caffeine exposure can alter salivary protein expression. Male Long Evans rats were given either a control diet or a diet containing 0.03% caffeine ad libitum. Animals on the caffeine containing diet decreased total intake, by decreasing the number of meals they consumed (p's<0.05) compared to controls. They also decreased their rate of feeding and meal size initially but increased these overtime (p=0.054, and p=0.008 respectively). Salivary protein expression after exposure to the caffeine diet at was upregulated in 14, 16, and 42 kilodalton (kDa) bands (p=0.019, p=0.014 and p=0.019 respectively) compared to controls. The 42kDa band was also correlated with change in rate of feeding in the caffeine treated animals (trend, p=0.07). Proteins in the 14 and 42 kDa bands have been previously linked to changes in taste responding. These data suggest that caffeine is able to drive changes in protein expression. Future work will focus on whether or not these proteins are, in turn, able to alter the taste of caffeine.

Disclosures: E. Demieri: None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.04/P4

Topic: E.03. The Chemical Senses

Support: Intramural Grant from NICHD

Title: Elucidating gustatory encoding by an interneuron in the Drosophila suboesophageal zone

Authors: *L. G. PAPPALARDO[1,2], M. A. STOPFER[1];
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Abstract: Gustation is essential for distinguishing foods from toxins and contributes to a positive quality of life. Yet, the neural mechanisms underlying gustatory signal processing remain elusive with competing hypotheses. Gustatory signals are thought to be encoded by labeled lines, with each "line" of neurons (from receptor cells to the brain) representing just one from a handful of basic tastes. Alternatively, gustation may be encoded through a combinatorial mechanism in which large numbers of individual tastes are represented by combinations of simultaneously active neurons. Drosophila melanogaster is a powerful model system that allows comprehensive and reproducible experiments to elucidate this mechanism at the level of single, identifiable neurons. The labeled line model predicts that information for different tastes remains segregated throughout the gustatory pathway, so lines representing different tastes should not converge. To test whether multiple types of first-order gustatory receptor neurons (GRNs) converge onto a single second-order interneuron in the subesophageal zone (SEZ), we employed both anatomical and physiological approaches focusing on the SEZ interneuron known as G2N-1 that we previously identified (Miyazaki et al., 2015).
First, by analyzing the EM connectome of the adult fly brain (Dorkenwald et al., 2024; Schlegel et al., 2024), we identified GRNs providing inputs to G2N-1 that are consistent with neurons expressing Gr64f (sugar-sensing receptor) and ppk28 (water-sensing receptor). Using the anatomical tracing method BacTrace (Cachero et al., 2020), we further found that neurons expressing ppk23 (salt-sensing receptor) and Gr5a (sweet-sensing receptor) also connect to G2N-1. Currently, we are using in vivo calcium imaging techniques to determine the range of tastants that functionally activate G2N-1.

Disclosures: L.G. Pappalardo: None. **M.A. Stopfer:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.05/P5

Topic: E.03. The Chemical Senses

Title: An evolutionarily conserved role of otopetrin channels in water detection underlies water satiety

Authors: *A. GANGULY[1], S. SWAMINATHAN[2], C. MONTELL[4], A. CHATTERJEE[3];
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Abstract: Water satiety, the cessation of drinking once hydration needs are met, is essential for maintaining fluid homeostasis and preventing overconsumption of water, which can result in life-threatening conditions such as water intoxication and hyponatremia. Thirst quenching can be triggered both by the detection of water through peripheral sensory neurons and by internal physiological changes following water absorption. Although central mechanisms of water homeostasis have been studied extensively, the neuronal and molecular bases of thirst quenching driven by sensory detection of water in peripheral taste organs remains poorly understood. Here, we identify *OtopLA*, a *Drosophila* homolog of the Otopetrins—an evolutionarily conserved family of proton channels—as a critical component of the peripheral sensory system mediating water satiety. *OtopLA* mutants show prolonged drinking bouts and a higher likelihood of initiating drinking, suggesting impaired water-satiety feedback. Restoring *OtopLA* in D (also known as high-salt) gustatory receptor neurons (GRNs) rescues normal drinking duration. Electrophysiological recordings reveal reduced water-evoked action potentials in D GRNs in *OtopLA* mutants, which are restored when *OtopLA*$^+$ is re-expressed in these neurons. Silencing D GRNs by expressing an inwardly rectifier potassium channel also increases drinking duration, further emphasizing the role of these neurons in water satiety. Ectopic expression of a known water receptor (*ppk28*) or mouse *Otop1* in all *OtopLA*-expressing cells restores both normal drinking duration and water-evoked activity in D GRNs, highlighting an evolutionarily conserved role for Otopetrins in water sensing. Additionally, we demonstrate that *Ppk23*, a DEG/ENaC channel, is co-expressed in D GRNs, and functions in regulating water satiety. Restoring *OtopLA* in GRNs alone fails to correct the increased drinking initiation rate, whereas pan-*OtopLA* rescue is effective, suggesting roles for *OtopLA* extend beyond GRNs. *OtopLA* mutants also exhibit reduced resistance to dehydration and increased excretion under both water-satiated and rehydrated conditions. Analysis of existing single-cell transcriptome datasets and *in vivo* imaging shows *OtopLA* expression in Malpighian tubules, implicating roles for OtopLA in systemic water retention and osmoregulation. Our findings uncover a conserved role of Otopetrins in peripheral water detection and establish *OtopLA* as a key molecular component linking sensory input to water satiety and hydration state.

Disclosures: A. Ganguly: None. **S. Swaminathan:** None. **C. Montell:** None. **A. chatterjee:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.06/P6

Topic: E.03. The Chemical Senses

Support: Leon Levy Scholarship

Title: Sweet memories: neural representation of taste-memory

Authors: *S. BERMAN[1], D. SHOHAMY[2];
[2]Zuckerman Inst., [1]Columbia Univ., New York, NY

Abstract: When we recall a past episode, we typically bring back to mind vivid multisensory details, such as sights, sounds, smells, and flavors. It is well established that the hippocampus and other medial temporal lobe regions play a key role in episodic memory retrieval, yet many open questions remain about the involvement of sensory-specific cortical areas. Moreover, while anecdotes and art often describe the special capacity of taste to elicit memory retrieval, experimental studies focused on taste as a sensory experience in memory are lacking.
Here we sought to investigate taste-memory, combining functional magnetic resonance imaging (fMRI) with a cross-modal paradigm. We were particularly interestedin exploring the insula, which is the primary gustatory cortex, as a candidate region likely to play an important role in taste-memory. First, participants (n=22) viewed objects while tasting six flavors (from three taste categories) during a flavor encoding phase. Next, to encourage flavor retrieval, participants viewed the objects alone and were asked to recall the associated flavor properties. After the scan, participants tasted the flavors again and rated their sensory dimensions.
During encoding of object-flavor association, we found that the insula showed increased BOLD activation for all flavors. Moreover, we found that perceptually similar flavors elicited similar activation patterns, suggesting the insula may encode aspects of flavor identity. During retrieval, we found increased BOLD activity in the insula when people were cued to recall the flavor, even in the absence of any sensory flavor input. Our findings provide new insights into the role of the insula in both encoding and retrieval of sensory details of taste, advancing our understanding of sensory representation in episodic memory.

Disclosures: S. Berman: None. **D. Shohamy:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.07/P7

Topic: E.03. The Chemical Senses

Support: CF-2023-G-518

Title: Acute semaglutide treatment enhances sweet taste perception

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Abstract: Organisms must distinguish gustatory from nutritional attributes to guide food intake. Hypercaloric diets contribute to obesity, a condition often treated with GLP-1 receptor (GLP1-R) agonists like semaglutide. While the weight-loss effects of semaglutide are well understood, its impact on taste perception and feeding decision-making remains incompletely characterized. This study evaluated the acute effects of semaglutide on sweet taste processing in C57BL/6 mice using two novel behavioral assays conducted via a custom-designed homegustometer, which allows continuous, automated home-cage behavioral monitoring. First, in a dynamic two-bottle preference test where glucose (18% w/v) and saccharin (0.1%) positions rapidly changed uncued based on lick counts (in blocks from 50, 100, 300, 500 and 1000 licks), mice had to continuously detect the sweetness of saccharin from the caloric content of glucose. Second, a water-glucose categorization task was used to assess psychophysical perception.A single administration of semaglutide induced an approximate 10% body weight loss in both sexes, which recovered within 72 hours. In the two-bottle test, semaglutide exerted an anorexigenic effect, reducing the total intake of both caloric and non-caloric sweetened solutions. Remarkably, despite this intake suppression, glucose-over-saccharin preference was unaltered, and the ability to discriminate glucose's caloric value from saccharin was enhanced during the first 24 hours post-administration. This suggests semaglutide does not impair caloric discrimination. Conversely, in the taste categorization task, semaglutide significantly reduced trial engagement and transiently impaired performance (56-60% accuracy) for approximately 18 hours, primarily by causing water to be miscategorized as sweet. Gustatory discrimination recovered in subsequent days.Neither essay indicated a conditioned taste aversion to glucose, suggesting that there was no actual effect on satiety. In summary, acute semaglutide strongly suppresses feeding and body weight. Furthermore, it has a complex impact on sweet taste processing, enhancing discrimination of caloric sweeteners while transiently altering basic sweet perception, leading to the misidentification of water as sweet. This work pioneers the characterization of the impact of semaglutide on taste perception.

Disclosures: O. Molina: None. **R. Gutierrez:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.08/P8

Topic: E.03. The Chemical Senses

Support: HHMI Freeman Hrabowski

Title: If You Give a Mouse a Cookie: Conditioned Taste Aversion in Parents and Innate Response in Offspring

Authors: S. GOLDBERG[1], ***C.-C. LEE**[4], N. MIMOUNI[2], W. FOSTER[2], C. LIFF[3], B. J. MARLIN[5];

[2]Neurosci., [3]Zuckerman Inst., [1]Columbia Univ., New York, NY; [4]Zuckerman Institute, Columbia Univ., New York City, NY; [5]Columbia Univ. Zuckerman Inst., New York, NY

Abstract: Associative learning allows animals to adaptively modify behavior in response to environmental cues. Conditioned taste aversion (CTA) is one such form, in which animals avoid a palatable stimulus after it is paired with an aversive outcome. While CTA is well characterized in conditioned individuals, whether such learned responses influence subsequent generations remains unclear. Here, we used a lithium chloride (LiCl)-based CTA paradigm to examine intergenerational epigenetic inheritance. Mice (F0) developed robust aversion to a sweet tastant following LiCl-induced malaise. High-speed videos revealed that conditioned mice exhibited behavioral responses resembling those of unconditioned mice exposed to bitter stimuli. To assess inheritance, conditioned males were mated with naïve females and removed before offspring were born to eliminate social transmission. Offspring (F1) of conditioned males do not show reduced preference for sweet tastants despite no prior exposure to conditioning. However, highspeed videos reveal nausea-like behaviors in the F1, compared to naïve controls. Moreover, preliminary data suggest LiCl training shifts inflammatory markers in the F1. These findings suggest that CTA learning can alter taste behavior, and physiological responses across generations, implicating mechanisms in the inheritance of aversive experiences.

Disclosures: S. Goldberg: None. **C. Lee:** None. **N. Mimouni:** None. **W. Foster:** None. **C. Liff:** None. **B.J. Marlin:** None.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

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Program #/Poster #: PSTR392.09/P9

Topic: E.03. The Chemical Senses

Support: NIH Grant 5R35GM152051-02

Title: Automated patch clamp assay for proton-activated hOTOP1 channel

Authors: J. KAPLAN[1], E. R. LIMAN[1], ***D. R. P. SAUTER**[2];
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Abstract: Manual patch clamp, invented in the late 1970s, remains the gold standard for studying ion channels due to its capability to directly measure ion channel function. However, this technique is low-throughput, labor-intensive, and requires a high level of expertise. Automated patch clamp (APC) systems address these limitations by enabling higher throughput and standardized recordings. The QPatch Compact (QPC) is an 8-well APC platform, featuring microfluidic flow channels, which allows for rapid extracellular solution exchange—an essential requirement for studying proton-activated ion channels. OTOP1 (Otopetrin 1) is a proton-selective ion channel expressed in many cell types and tissues, including the inner ear and taste receptor cells, where it plays a critical role in otoconia formation and acid sensing. In taste cells,

OTOP1 functions as a sour taste receptor and ammonium sensor, transducing changes in pH and ammonium into action potentials that signal to gustatory nerves. OTOP1 is gated by extracellular pH changes and exhibits high proton selectivity, allowing it to mediate responses to acidic environments. Understanding OTOP1's biophysical properties is crucial for elucidating its physiological roles and therapeutic potential. We have developed an assay to study human OTOP1 recombinantly expressed in HEK293 cells using the QPatch Compact platform. Our recordings demonstrate that OTOP1 currents can be robustly activated by extracellular protons in a dose-dependent manner, confirming the channel's pH-dependent activation. Additionally, application of extracellular ammonium evoked inward proton currents, consistent with OTOP1's role as a sensor for ammonium. Furthermore, we confirmed that Zn^{2+} inhibits human OTOP1, demonstrating that this assay can be used to evaluate novel channel inhibitors. This study serves as a proof of principle that OTOP1 can be reliably recorded using the QPatch Compact system. Our assay is well-suited for identifying novel modulators of OTOP1 that could facilitate future investigations into its physiological role and used for therapeutic applications.

Disclosures: J. Kaplan: None. **E.R. Liman:** None. **D.R.P. Sauter:** A. Employment/Salary (full or part-time): Sophion Bioscience.

Poster

PSTR392: Taste

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR392.10/P10

Topic: E.03. The Chemical Senses

Title: Artificial sweeteners differentially activate sweet and bitter gustatory neurons in Drosophila melanogaster

Authors: *C. ARNTSEN[1], M. STANLEY[2];
[2]Biol., [1]Univ. of Vermont, Burlington, VT

Abstract: Artificial sweeteners are highly sweet, non-nutritive compounds widely used to reduce caloric intake while maintaining palatability. While these additives are considered biologically inert and safe for consumption, their long-term health impacts remain unclear and controversial. The distinctive chemical properties of these sweeteners, which are hundreds to thousands of times sweeter than sucrose, have raised questions about how the gustatory system encodes these compounds. Emerging evidence suggests that these chemicals also interact with bitter taste receptors, implying that artificial sweeteners represent a more complex chemosensory signal. The fruit fly, *Drosophila melanogaster*, serves as a valuable model organism for investigating the effects of artificial sweetener exposure on many phenotypes. However, the basic chemosensory properties of these chemicals, and their influence on feeding, remain poorly understood. Therefore, we assessed how flies responded to a panel of commonly used artificial sweeteners and found concentration-dependent responses to the different tastants. Using *in vivo* calcium imaging, we next recorded sweetener-induced activity in the axon terminals of sweet

and bitter gustatory receptor neurons (GRNs), two populations that reciprocally impact feeding behavior. We found that sucralose and rebaudioside A (Reb A) both co-activate sweet and bitter GRNs, while aspartame only activates bitter GRNs. To determine the behavioral impacts of sweet-bitter co-activation, we implemented a prolonged taste modulation paradigm to show that low concentrations of sucralose signal appetitive feeding while high concentrations generate an aversive "aftertaste" effect that reduces feeding. To dissect the neuronal basis of these effects, we used optogenetic and genetic silencing tools to manipulate GRN activity. Silencing sweet GRNs reduced behavioral attraction to low sucralose concentrations and abolished long-term appetitive feeding. In contrast, silencing bitter GRNs reduced the aversive signal elicited by high concentration sucralose and enhanced feeding behavior. Together, we conclude that artificial sweeteners generate a gustatory signal that is more complex than "sweetness" alone, and this bitter co-activation has behaviorally relevant effects on feeding that may help flies flexibly respond to these unique compounds.

Disclosures: C. Arntsen: None. **M. Stanley:** None.

Poster

PSTR392: Taste

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Program #/Poster #: PSTR392.11/P11

Topic: E.03. The Chemical Senses

Support: Howard Hughes Medical Institute

Title: The structure of human sweet receptor

Authors: *J. ZHANG, Z. LU, C. ZUKER;
Columbia Univ., New York, NY

Abstract: In humans, the detection and ultimately the perception of sweetness begins in the oral cavity, where taste receptor cells (TRCs) dedicated to sweet-sensing interact with sugars, artificial sweeteners, and other sweet-tasting chemicals. Human sweet TRCs express on their cell surface a sweet receptor that initiates the cascade of signaling events responsible for our strong attraction to sweet stimuli. Here we describe the cryo-electron microscopy structure of the human sweet receptor bound to two of the most widely used artificial sweeteners, sucralose and aspartame. Our results reveal the structural basis for sweet-detection, provide insights into how a single receptor mediates all of our responses to such a wide range of sweet-tasting compounds, and open up unique possibilities for designing a generation of taste modulators informed by the structure of the human receptor.

Disclosures: J. Zhang: None. **Z. Lu:** None. **C. Zuker:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.01/P12

Topic: E.06. Vision

Title: Comparison of task performance using licking- or running-report in operant visual discrimination task in head-fixed mice

Authors: *S. NISHIMOTO[1,3], M. WATANABE[2], A. FUNAMIZU[1,3];
[1]Grad. Sch. of Arts and Sci., [2]Grad. Sch. of Engin., The Univ. of Tokyo, Tokyo, Japan; [3]Inst. for Quantitative Biosci., Tokyo, Japan

Abstract: Visual discrimination tasks in head-fixed mice are essential for elucidating the neural basis of visual processing with high temporal and spatial resolution neural recordings and manipulations. Here, we investigated how different behavioral report paradigms (licking vs. deceleration) affect the task performance. Initially, mice were trained on a Go/No-Go task to discriminate white (Go) from black (No-Go) visual stimuli. Mice self-initiated trials by running, and received water rewards only if they licked a spout during a one-second window between 1 and 2 seconds after stimulus onset (reward window) in Go trials. In No-Go trials, mice had to withhold licking. After 10 sessions, no significant differences in licking behavior were observed between trial types: mice made an average of 2.8 licks with a first-lick latency of 0.58 seconds (6 mice, 12 sessions). Running speed also showed no significant difference between Go and No-Go trials. With continued training until reaching ≥80% accuracy in Go and ≥50% in No-Go trials, licking became more temporally aligned with the reward window, and the reaction time increased to 0.79 seconds. Within 1 second of stimulus onset, lick counts were significantly higher in Go than in No-Go trials ($p = 0.002$, signed-rank test). Interestingly, mice also showed greater deceleration in Go than No-Go trials, despite its apparent irrelevance to reward acquisition. Motivated by the observation of the change in running speed in a licking-based report paradigm, we developed a deceleration-based report paradigm, in which mice were rewarded for reducing running speed below 5 cm/s within the reward window. After 10 sessions of training, no clear stimulus-dependent behavioral differences were observed (6 mice, 12 sessions). However, compared to the lick group, mice showed fewer licks (2.1 on average) and longer first-lick latencies (0.72 seconds), suggesting reduced unnecessary licking. Continued training led to equivalent performance levels (≥80% correct for Go, ≥50% for No-Go), and the reaction time increased further to 0.92 seconds. Go trials elicited significantly more licking than No-Go trials ($p = 0.02$, signed-rank test). Notably, deceleration was more pronounced in Go trials than in No-Go trials, and this difference was larger than that observed in the lick group ($p = 0.006$, Mann-Whitney U test). These findings demonstrate that the modality of behavioral reporting shapes performance in visual discrimination tasks. The deceleration paradigm elicited stronger behavioral biases between Go and No-Go stimuli across multiple metrics than licking paradigm, suggesting it may offer improved sensitivity for probing perceptual decisions.

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Poster

PSTR393: Visual System: Responses During Behavior

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Title: State-dependent reconstruction of natural images from mouse visual cortex

Authors: *L. XU[1], A. HAYDAROGLU[2], M. SHINN[1], S. SKRIABINE[1], K. D. HARRIS[1], M. CARANDINI[1];
[2]Inst. of Neurol., [1]Univ. Col. London, London, United Kingdom

Abstract: The encoding of images by neurons in the visual cortex is known to depend on a variety of factors, including brain state. In mice, visual responses differ when the animal is running versus stationary (Niell and Stryker, 2010; Pakan et al., 2016), raising the question of how brain state influences the representation of complex, real-world visual input. To address this, we used an image reconstruction approach, in which natural images are reconstructed from large-scale neural activity in the visual cortex. This method provides an approximation to assess how much information about the visual input is contained in neural signals, and whether this information differs between behavioral states. We recorded two-photon calcium activity from >15,000 neurons from the visual cortex of awake, head-fixed mice (n = 2) while presenting grayscale natural images spanning 90° × 67.5° of visual angle. Images were presented in randomized blocks, and we divided the data into training (1,797 stimuli, ×2 trials each) and test sets (63×8). Each image was transformed into a Gabor feature representation at multiple spatial scales, following Yoshida and Ohki (2020). We used ridge regression to learn a linear mapping from neuronal activity to Gabor features and then reconstructed the corresponding images. We then compared reconstruction performance between running and stationary periods to assess the effect of behavioral state. The model achieved a mean pixel-wise correlation of 0.46 ± 0.18 for Mouse 1 and 0.47 ± 0.19 for Mouse 2 on the test set when using coarse Gabor features (spanning ~30° of visual field), indicating reliable reconstruction of global image structure. Performance decreased with finer spatial features (0.26 ± 0.17 for Mouse 1 and 0.39 ± 0.18 for Mouse 2), likely due to model's simplicity rather than a lack of encoding, as many of the neurons were well-tuned to finer features (~8°). Crucially, reconstruction performance improved significantly during running compared to stationary periods. The mean pixel-wise correlation had a statistically significant increase by 0.09 in Mouse 1 and by 0.11 in Mouse 2 during running,

suggesting a strong modulation of visual representations by behavioral state. Our findings demonstrate that linear decoders can reconstruct natural images from mouse visual cortex activity, with better performance for coarse features, providing an approximation of visual representations. Importantly, reconstruction performance was significantly enhanced during running, highlighting the role of arousal or cortical state in shaping sensory representations.

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Poster

PSTR393: Visual System: Responses During Behavior

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Title: Eye-tracking to correct gaze shifts in a natural virtual reality system for recording mouse visual cortical activity

Authors: *D. AU[1,2], **Z. ALAOUI**[2], J. B. MELANDER[3], J. C. WEDDINGTON[3], Y. FARAGALLA[3], S. LIU[4], S. A. BACCUS[2];
[1]Neurobio., Stanford Univ., Palo Alto, CA; [2]Neurobio., [3]Neurosci. Program, [4]Applied Physics, Stanford Univ., Stanford, CA

Abstract: Studies of the mammalian visual system often involve stimulus presentation to head-mounted animals to ensure precise control of visual input and to maintain stability for electrophysiology or optical recording. However, in natural vision, the retinal image constantly shifts due to saccadic and fixational eye movements. Although these gaze shifts are routinely measured in primates, they are often not accounted for in mice. Here, we present an eye-tracking method for head-fixed mice that enables precise, frame-by-frame measurement of gaze shifts during naturalistic visual stimulation. Using an IR camera and IR LEDs placed at known locations on the stimulus screen, we record the 3D positions of the eye, camera, and LEDs to establish coordinate systems for the monitor and camera (3D Cartesian), the eye (spherical), and the image (2D cartesian), along with transformations between those coordinates. To estimate gaze direction, we apply custom computer vision algorithms to extract the 2D pupil center and reference LED reflections in each video frame. A vector for the center of gaze in eye coordinates is computed for each frame using the pupil and a fiducial LED and the camera, and then that

gaze vector is traced to a monitor pixel. These dynamic gaze angles are then used to remap each frame of the visual stimulus into gaze-aligned (retina-centered) coordinates, capturing the visual input as it appears on the retina. Our method enables detailed analysis of saccadic eye movements and gaze variability under different stimulus conditions, including naturalistic videos, drifting gratings, and white noise patterns. Using convolutional neural network models trained on gaze-aligned stimuli, we demonstrate improved predictive accuracy of neural responses compared to models trained on traditional uncorrected monitor input. Furthermore, this approach yields smaller and more structured receptive field maps, suggesting previous methods may underestimate the precision of cortical receptive field structure. Our eye-tracking approach offers a powerful tool for investigating active visual sensing in mice under natural viewing conditions and provides a framework for incorporating dynamic gaze information into computational models of visual processing.

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Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

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Title: Locomotion modulates visual adaptation in the mouse retino-collicular circuit

Authors: *M. GONZÁLEZ FLEITAS, R. GABDRASHOVA, L. J. BARUCHIN, S. SCHRÖDER;
Sch. of Life Sci., Univ. of Sussex, Brighton, United Kingdom

Abstract: Visual coding continuously adapts to behavioral and sensory contexts. While emerging evidence shows that behavioral states such as locomotion and arousal shape visual response strengths even at the level of the retina, the influence of state on temporal dynamics remains poorly understood. Here, we aim to understand how locomotion impacts adaptation to continuous visual stimulation in the superior colliculus (SC), a key visual area that integrates input from the retina and primary visual cortex (V1) in its superficial layers and performs sensorimotor transformations in its deeper layers.We recorded single-unit responses in the SC using acute extracellular electrophysiology in awake head-fixed mice free to run at will. Visual adaptation was assessed using drifting sinusoidal gratings in 12 directions, interleaved by a gray screen. Trials were labelled as “locomotion” or “stationery” based on running speed being above or below 1cm/s for 90% of the trial duration. Most neurons (68%) in the superficial SC exhibited

one of two adaptation dynamics: (a) strong transient response followed by quick adaptation or (b) sustained response adapting slowly. Similar temporal patterns were observed in the deep SC (49%). However, more neurons often showed no visual adaptation (40%) or increasing activity during stimulus presentation (11%). Locomotion significantly modulated adaptation strength in approximately 25% of visually driven SC neurons, generally leading to stronger reduction of firing rate across time. Locomotion was also associated with faster adaptation indicated by reduced time constants in exponential fits. These locomotion-induced changes were similarly distributed across superficial and deep SC.To determine whether these effects originate in the retina, we isolated retinal axon activity from cell bodies and cortical axons based on spike waveform and visual responsiveness to drifting gratings using tangentially inserted silicon probes. Silencing neurons in the SC and V1 confirmed this approach. Retinal axons showed weaker adaptation than somatic units in superficial SC, but locomotion significantly increased adaptation magnitude in around 20% of visually responsive axons. State modulation of adaptation thus begins in the retina. Importantly, the effects of locomotion on adaptation in both retinal axons and SC neurons were only weakly correlated with effects on mean firing rate.Our findings demonstrate that locomotion enhances adaptation strength and speed in the SC and its retinal input, providing novel insights into how behavioral states shape sensory processing from the retina and across the visual hierarchy.

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Poster

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Topic: E.06. Vision

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Title: Tunnel motion triggers looming responses in mice

Authors: *A. HASHIO[1], S. NAKAMURA[1], H. SHUTO[1], A. KITAOKA[2], C. KOIKE[3]; [1]Ritsumeikan Univ., Kusatsu-shi, Shiga, Japan; [2]Ritsumeikan Univ., Ibaraki-shi, Osaka, Japan; [3]Ritsumeikan Univ., Kusatsu, Japan

Abstract: Tunnel vision is the perceptual illusion of moving into a narrowing tunnel or dark hole, triggered by both dynamic and static visual patterns. In humans, pupil dilation has been selectively observed in response to illusory stimuli in which a dark central region appears to expand, potentially reflecting an adaptive mechanism that anticipates entry into a darker environment (Laeng et al., 2024). However, it remains unknown whether similar perceptual

responses occur in nonhuman species, such as mice. To date, whether mice perceive and behaviorally respond to visual illusions has been addressed in only a few studies, such as one on the Kanizsa illusion (Pak et al., 2020), leaving this area largely unexplored. Thus, investigating whether visual illusion stimuli elicit innate responses in mice is a compelling topic in vision science. Here, we investigated whether a specific type of visual illusion stimulus—derived from the tunnel vision illusion—can trigger innate looming-escape responses in mice. Looming stimuli are rapidly expanding black discs that mimic an approaching predator and elicit instinctive escape behaviors in rodents (Yilmaz & Meister, 2013).We first optimized experimental conditions to reliably induce escape behavior in awake, freely moving C57BL/6J mice. Under control conditions, the average escape rate to real looming stimuli was 16.7%. By adjusting housing conditions, we increased this rate to 86.7% under luminance levels comparable to the visual illusion stimuli. Under these optimized conditions, the average escape rate to the visual illusion stimuli was 28.3%, which was significantly higher than the response to control stimuli (10.8%) (One-factor ANOVA with three levels, $p < .05$). These results demonstrate that innate defensive behaviors can be triggered even by stimuli that lack actual physical expansion. Our findings suggest that the retinal looming detection circuit—including W3 and tOFFα retinal ganglion cells receiving feature-selective input from VGLUT3-expressing amacrine cells and modulated by TH2 and AII amacrine cells (Kerschensteiner, 2022)—can be activated not only by real expanding stimuli but also by perceptual illusions. Alternatively, these results may point to the existence of yet unidentified visual detection pathways capable of responding to ambiguous or illusory threats, offering a promising direction for future research.

Disclosures: A. Hashio: None. **S. Nakamura:** None. **H. Shuto:** None. **A. Kitaoka:** None. **C. Koike:** None.

Poster

PSTR393: Visual System: Responses During Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.06/Q5

Topic: E.06. Vision

Support: NIH UF1 NS116377
NIH R01 NS118457
AFOSR 19RT0316
Kavli Institute for Brain and Mind Postdoctoral Award

Title: The role of active vision in the primary visual cortex of freely-moving marmosets

Authors: *J. LI[1], J. F. MITCHELL[2], A. HUK[3], C. T. MILLER[4];
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Abstract: Historically, studies of visual cortex have been performed while nonhuman primates are head-fixed viewing visual stimuli on a screen. In the real world, however, visual processing must accommodate how we actively explore the environment. Despite its significance, little is known about how the primate visual system supports natural, active vision in freely moving animals. To address this problem, we leveraged an innovative, head-mounted eye-tracking system developed for marmosets in our lab while simultaneously recording the activity of single neurons in V1 to examine the effect of eye, head, and body movement on visual representations. In these experiments, monkeys are first head-fixed to quantify traditional visual stimuli for receptive field and tuning properties, and then brought to freely explore a large arena where high-contrast visual stimuli are shown on the wall. We first successfully recapitulated the receptive fields and turning properties of the V1 neurons in the head-restrained scenario. In the freely-moving scenario, we found that the primate V1 population consistently response to gaze shift and fixation with a suppression followed by an enhancement in a sequential latency. Moreover, we found that the suppression is associated with gaze-shift onset, whereas the enhancement is associated with gaze-fixation onset. A model of gaze response built upon this mechanism well recapitulates the observed data. The effect of locomotion, however, modulates the baseline of neural activity and is speed correlated. To further dissect the neural responses driven by visual input and movement, we performed the experiment in the dark condition. Surprisingly, most responses to gaze movement are disrupted and the effect of locomotion is no longer significant in the dark. Preliminary analyses of visual scenes show a different statistic of visual input induced by locomotion, influencing V1 neurons differently depending on their turning properties. These data are the first to examine the neural basis of active vision in a freely-moving primate and have a significant influence on our conceptions of natural vision.

Disclosures: J. Li: None. **J.F. Mitchell:** None. **A. Huk:** None. **C.T. Miller:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Program #/Poster #: PSTR393.07/Q6

Topic: E.06. Vision

Support: NIH Grant U01MH130907

Title: Bridging Function and Transcriptomics: Visual Responses of Diverse Inhibitory and Excitatory Cell Types in the Mouse Visual Cortex

Authors: *O. ZOBEIRI[1], H. SCHRYVER[2], J. CAMPOS[1], J. KIM[1], C. BERRY[1], S. MCCULLOCH[1], N. MARTIN[4], P. OLSEN[4], A. WILLIFORD[1], S. M. SEID[5], M. S. ALOI[3], P. A. GROBLEWSKI[6], D. MCMILLEN[1], S. E. DEVRIES[7], S. R. OLSEN[1], J. WATERS[6], M. E. GARRETT[8], A. ARKHIPOV[1];
[2]Modeling & Theory, [3]Psychiatry, [1]Allen Inst., Seattle, WA; [4]Allen Inst. for Brain Sci., Seattle, WA; [5]Allen Inst. For Brain Sci., Kent, WA; [6]Allen Inst. For Brain Sci., Seattle, WA; [7]Allen Inst.

For Neural Dynamics, Seattle, WA; [8]Neural Circuits and Behavior, Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: The intricate functions of the brain arise from diverse populations of neurons, each with distinct genetic profiles and potentially unique roles in neural circuits. A central question in neuroscience is how these diverse cell types contribute to the broader functional properties of neural circuits. To elucidate this complex interplay, we are investigating the patterns of activity generated by different cell types in the visual cortex when processing visual information and generating behavior.In our experiments, we present mice with various visual stimuli, such as moving gratings and natural movies, while monitoring neural activity in the visual cortex. We employ multiplane two-photon calcium imaging in vivo to record neural responses, followed by both thick- and thin-tissue approaches for post-hoc spatial transcriptomics, facilitating the mapping of single cells across different modalities, linking transcriptomic identity to neural response. The thick-tissue approach involves multi-round hybridization chain reaction (HCR) and multi-channel light-sheet imaging, whereas in the thin-tissue approach, the Xenium In Situ platform (10x Genomics) is used after serial sectioning of the tissue.We have successfully co-registered functional data with spatial transcriptomics, revealing neural responses to visual stimuli consistent with data from subclass-specific transgenic mice. In addition, preliminary results indicate distinct clusters of neurons based on gene expression, with specific patterns emerging even with a limited number of genes. Our findings suggest a correlation between transcriptomic supertypes and functional responses, with notable diversity observed within subclasses such as Pvalb.Future research directions include expanding the dataset and investigating additional functional characteristics of the responses to natural movies or behaviors.

Disclosures: O. Zobeiri: None. **H. Schryver:** None. **J. Campos:** None. **J. Kim:** None. **C. Berry:** None. **S. McCulloch:** None. **N. Martin:** None. **P. Olsen:** None. **A. Williford:** None. **S.M. Seid:** None. **M.S. Aloi:** None. **P.A. Groblewski:** None. **D. McMillen:** None. **S.E. DeVries:** None. **S.R. Olsen:** None. **J. Waters:** None. **M.E. Garrett:** None. **A. Arkhipov:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

Support: NEI: 1R01Ey032101 (J.L.H)
NIGMS: P20GM103650 (COBRE)
NIH: GM103440 (INBRE)

Title: Visual motion perception is altered in adolescent Shank3B mice

Authors: K. M. ALLEN;
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Abstract: Autism spectrum disorder (ASD) is a prevalent neurodevelopmental condition characterized by a variety of neurobehavioral challenges, including irregularities in visual perception. However, it is unknown if the etiology of behavioral abnormalities in ASD may be due to anomalies in early developmental visuomotor mechanisms. Though phenotypically and genetically heterogeneous, Shank3 is a primary candidate gene underlying the etiology of ASD. Shank3KO mouse models provide evidence of behavioral similarities exhibited by humans with autism spectrum, such as repetitive behaviors and decreased efficiency in goal-directed actions. However, it remains unclear if abnormalities in visual processing contribute to these behavioral differences in mouse models of autism. To address this, we quantified adolescent Shank3B mice's natural visual behavior towards our computerized-spontaneous perception of objects task (C-SPOT). We found robust genotype-dependent differences in visual behavior, governed by genotype-dependent stimulus feature differences. Overall, we are working to understand visual processing changes in mouse models of autism and how visual perceptual differences lead to behavioral differences in those with autism.

Disclosures: K.M. Allen: None.

Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

Support: Sir Henry Dale fellowship with Wellcome Trust to R.S. (grant code: 220163/Z/20/Z)

Title: Postural perturbations induce fast multimodal integration in the mouse visual thalamus

Authors: *R. STORCHI[1], A. EBRAHIMI[2];
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Abstract: A common problem that most brains have to face is postural control. Walking on unstable or slippery terrain, climbing on trees, being pushed by someone or something are all examples of perturbations that induce passive or unintended movements and challenge animal's balance. In such conditions the temporal frame in which brains integrate vision and movements is imposed by the temporal dynamics of the perturbation rather than controlled by the subject. Which stages of the visual system react to postural perturbations and the nature and temporal dynamics of these responses is largely unknown. Since postural perturbations require quick multimodal integration, fast temporal alignment between vision and movement is a key factor. We hypothesize that the dorsal Lateral Geniculate Nucleus (dLGN), receiving cortical feedback,

visual information from the retina and nonvisual information from the brainstem, plays a role in this process. To test this hypothesis, we developed a behavioural assay in which mice, implanted with multichannel recording electrodes, are placed in a motorised arena, and postural perturbations are delivered by controlled arena tilts. We found that tilts evoked responses in a sizable fraction of dLGN cells, overwhelmingly represented by transient increments in firing rate. This effect was also observed in complete darkness, confirming its nonvisual nature. Simultaneous responses to tilt and to changes in illumination interacted additively, inducing a global shift in visual signalling. By combining 3D tracking of the animal and the arena we geometrically separated active movements (AMs) expressed by the animal from passive movements (PMs) induced on the animal by the arena tilts. A GLM analysis revealed that dLGN cells encode a combination of AMs and PMs with higher weights assigned to AMs while neighbouring subcortical regions weighted AMs and PMs more evenly. Making the timing of the tilt event fully predictable and increasing baseline arousal before the tilt did not qualitatively affect these results. Repeating these experiments in anaesthetised animals preserved light responses but largely abolished the effects of the tilts. These results highlight the flexible nature of multimodal integration, suggesting that, under tight temporal constraints, first order sensory thalamic nuclei, traditionally considered unimodal regions, could play a key role in coordinating multiple streams of sensory and motor information.

Disclosures: R. Storchi: None. **A. Ebrahimi:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

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Gilbert Family Foundation
Knights Templar Eye Foundation
Research to Prevent Blindness, Inc.

Title: Vision improvement without neuroprotection in a mouse model of NF1

Authors: *B. K. YOUNG[1], K. BEHERA[1], A. M. BLUEBELL[1], J. L. GOLDBERG[2]; [1]Ophthalmology, [2]Byers Eye Institute--Ophthalmology, Stanford Univ., Palo Alto, CA

Abstract: Purpose: Neurofibromatosis type-1 (NF1) is a genetic disorder caused by mutations in the neurofibromin tumor suppressor gene. 15-20% of patients with NF1 form optic pathway gliomas (OPGs), which frequently result in visual deficits due to damage to retinal ganglion cells (RGCs) whose axons travel through the optic nerve. Current treatments for vision loss in NF1 have poor outcomes; therefore, new therapies are needed. Here we describe our efforts to

evaluate and treat vision loss in a mouse model of NF1. **Methods:** We crossed two mouse lines, one that is hemizygous for NF1 (NF1KO/fl) with another that expresses GFAP:Cre in astrocyte precursor cells at embryonic day 11.5 (NF1KO/fl; GFAP:Cre). This double knockout of NF1 in astrocyte precursors leads to glioma formation in the optic nerve ("NF1-OPG mice"). Only female mice were used for this study due to their increased RGC loss. To evaluate vision, we used optomotor reflex to measure visual acuity, pupillary light reflex to evaluate light sensitivity, and looming object fear response to assess contrast detection. Immunofluorescence staining was used in retinal tissue to identify cell types and numbers. In a masked experiment, we injected either a control viral vector (AAV2-mSncg-Luciferase) or constitutively active calcium/calmodulin-dependent protein kinase II (AAV2-mSncg-CaMKiiα) at P30-P50. Eleven littermate controls (NF1fl/fl) and seven NF1-OPG were used in each group. **Results:** By postnatal day 120, NF1-OPG mice have severe visual defects in both looming and optomotor reflex response; however, the pupillary light reflex is mostly intact. Immunofluorescence labeling of RGCs showed 16% lower RGC density with some regions of the retina having over 50% loss in NF1-OPG mice. We found a significant defect in the spatial frequency acuity in NF1-OPG mice in the AAV2-mSncg-Luciferase-treated control group. In contrast, after treatment with AAV2-mSncg-CaMKiiα there was no significant reduction in visual acuity in NF1-OPG mice (0.25 ± 0.02 vs. 0.18 ± 0.02 cycles/degree, $p = 0.7419$, Kruskal-Wallis test). Despite this protection against functional vision loss, there was no difference in RGC density between control and CaMKiiα-treated NF1-OPG mice (2890 ± 78 vs 2846 ± 100 RGCs/mm2, $p = 0.9996$, Šidák test). **Conclusions:** Visual deficits in NF1-OPG mice can be prevented with early treatment with constitutively active CaMKii gene therapy, even without neuroprotection of RGCs, suggesting vision loss is due to additional disruptions in neural signaling or circuitry. Therefore, effective strategies for improving visual function in NF1 patients must consider the role that NF1 plays in neuronal signaling.

Disclosures: B.K. Young: None. **K. Behera:** None. **A.M. Bluebell:** None. **J.L. Goldberg:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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AFOSR 19RT0316
Kavli Institute for Brain and Mind Postdoctoral Award

Title: Effect of locomotion on the primary visual cortex of freely-moving marmosets

Authors: *S. EDLABADKAR[1], J. LI[2], C. T. MILLER[3];
[1]Computer Sci. and Engin., UCSD, La Jolla, CA; [2]Psychology, UC San Diego, San Diego, CA; [3]Cortical Systems and Behavior Lab., Univ. of California San Diego, La Jolla, CA

Abstract: Historically, studies of primate visual systems have largely relied on head-fixed paradigms. In real-world contexts, however, natural vision in primates requires the integration of sensory input with self-generated movements to actively decide when and where to go. Locomotion, as a way of purposely navigating through the environment, generates statistics and dynamics of visual inputs that are different from sitting and scanning. Moreover, it introduces potential non-visual signal modulation on the visual cortex. To investigate how locomotion influences neural activities in the primary visual cortex (V1), we recorded local field potentials (LFPs) in V1 simultaneously with precise body tracking of freely-moving marmosets. In these experiments, subjects were placed in a large arena to freely explore the environment. The behavior of subjects was tracked by a top-down camera and annotated with SLEAP. We first asked whether V1 exhibits behaviorally relevant activity patterns in specific frequency bands associated with locomotion. Spectral analysis of LFPs showed that V1 activity had an increased power in gamma (30-80 Hz) and beta (12-30 Hz) bands during locomotion. These bands also provided the best decoding performance of the locomotion state using a lightGBM classifier. Furthermore, temporal analysis of the power spectrum revealed that locomotion onset produced a phase reset in theta (4-7 Hz), alpha (8-12 Hz), and beta bands, indicating a reorganization of low-frequency oscillations. These findings suggest that primate V1 engages distinct frequency-specific dynamics during active exploration and locomotion, potentially serving to establish the optimal sensory encoding in real-world contexts. Together, this work provides the first insight into the neural mechanisms that link locomotion and visual processing in V1 of freely-moving primates during active exploration.

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Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

Support: National Science and Technology Innovation 2030 Major Program (2022ZD0204600)

Title: Neural mechanism of binocular and interocular rivalry revealed by two-photon and high-speed widefield calcium imaging in macaque visual cortex

Authors: *H. DING, M. LI;
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Abstract: There have been numerous studies on the neural mechanisms of binocular rivalry and interocular rivalry. However, many of these studies still present contradictions, and due to technical limitations, there is currently a lack of direct evidence to support them. We trained macaques to report the perceptual states and used two-photon and high-speed wide-field calcium imaging to measure the neural activity in V1 and V2. The behavioral results indicated that macaques could reliably report the states of binocular rivalry and interocular rivalry. In binocular rivalry trials, the proportion of reporting the complete rivalry state was 60.77%; in interocular rivalry states, the proportion of reporting the grouping rivalry state was 54.63%. While the physical switching reporting accuracy was 91.48%. By analyzing the activation of different functional columns in V1 and V2, we found that neural activity was correlated with behavioral data. Our current data suggest that binocular and interocular rivalry signals exist in the early visual cortex.

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Poster

PSTR393: Visual System: Responses During Behavior

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Program #/Poster #: PSTR393.13/Q12

Topic: E.06. Vision

Title: Visual cortex in context : how cognitive states influence cortical activity in response to dynamic naturalistic input

Authors: *S. PATEL[1,2], M. ABD EL HAY[1], A. TLAIE BORIA[1], K. SHAPCOTT[1], P.-A. FERRACCI[1], M. BANOU[1], M. N. HAVENITH[1], M. L. SCHÖLVINCK[1];
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Abstract: Our understanding of the visual system has been shaped primarily by studies relying on simplified stimuli (e.g., gratings), controlled cognitive states (e.g., sustained attention), and constrained behaviors (e.g., button presses). While this approach has uncovered many foundational principles, initial evidence shows that more naturalistic input alters even core concepts like the receptive field (RF) [1]. This raises questions about the completeness of our knowledge. In everyday life, the visual cortex operates within a dynamic feedback loop, integrating constantly changing visual input with ongoing cognitive processes. However, how naturalistic inputs and internally generated cognitive states interact to shape visual processing remains largely unknown. To investigate this interplay under ecologically valid conditions, we trained two monkeys to perform an intuitive foraging task in a naturalistic virtual reality (VR) environment projected onto the interior of a large dome [2]. The task was designed to engage a broad range of cognitive states, which were not experimentally imposed but spontaneously generated by the monkeys. While head-fixed, the monkeys could freely move their eyes and

navigate the VR using a trackball. Behavior was continuously tracked via multiple cameras capturing facial expressions and eye movements. To model internal state dynamics during this task, we applied a data-driven Markov-Switching Linear Regression (MSLR) framework [3] to this rich behavioral dataset. Thereby we inferred latent internal states corresponding to cognitive processes like attention or engagement, on a single-trial basis. In the poster, we will examine how neural activity in V1 and V4 (via six chronically implanted 32-channel arrays) varied with these cognitive states, particularly when the RF is aligned with behaviorally relevant input. This will help us understand how continuous cognitive states play a role in shaping processing in the early visual cortex under conditions where visual input is not tightly controlled. By combining immersive VR, rich behavioral monitoring, and latent state modeling, our approach provides a flexible framework to study how cognitive dynamics shape neural responses in real-world conditions. **References** [1] Vinje and Gallant, Science 2000 [2] Shapcott et al, PLoS One 2025 [3] Tlaie et al, Nat Comm 2025

Disclosures: S. Patel: None. **M. Abd El Hay:** None. **A. Tlaie Boria:** None. **K. Shapcott:** None. **P. Ferracci:** None. **M. Banou:** None. **M.N. Havenith:** Other; part of 3DNeuro. **M.L. Schölvinck:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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NIH Grant R01EY034723
NIH Grant RF1NS121913

Title: Coordinated Activity in V4 and 7a Enables Few-shot Generalization to Novel Shapes

Authors: *R. SRINATH, M. R. COHEN;
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Abstract: We readily and rapidly generalize our knowledge of object features to novel objects. For instance, we easily apply knowledge about picking familiar fruits like apples and bananas to less familiar starfruit. The neural basis of this remarkable, few-shot generalization ability has been challenging to pinpoint because laboratory experiments typically limit the range of stimuli and focus on behaviors that animals find challenging to generalize.
We trained monkeys to flexibly report the axial curvature of any randomly generated 3D shape on a continuous scale. The monkeys were trained to report the curvature of a subset of shapes.

During experiments, we compared their judgments of familiar shapes, variations of familiar shapes that differed only in orientation or color, as well as entirely novel shapes. We investigated the neural basis of few-shot generalization in the responses of simultaneously recorded populations of neurons in visual areas V1 and V4 and parietal area 7a. Behaviorally, we found that monkeys made several estimation errors when first encountering a novel shape, a hallmark of few-shot generalization. These initial errors lacked a smooth, predictable pattern and were not explained by the perceptual similarity between the novel and familiar shapes.
We tested two hypotheses about the potential neural underpinnings of these initial errors and rapid generalization -- (1) that downstream areas in the brain rapidly learn to interpret the fixed visual responses of these novel objects by changing the way those signals are read out to guide choices, and (2) that the visual responses to novel objects rapidly change (e.g., through repetition suppression, adaptation, or other modulatory processes) in ways that are conducive to an ongoing, fixed readout strategy. We found strong support for the second hypothesis. The visual responses in area V4 during early error trials were further separated from stable, 'learned' responses compared to later errors which were matched in magnitude. Concurrently, this separation in V4 was significantly correlated with the activity in parietal area 7a. In contrast, there was no relationship between 7a activity and V4 responses during erroneous judgments about familiar shapes. These results suggest parietal area 7a reflects signals that guide changes in visual cortex that enable few-shot generalization to novel shapes. This coordination between the visual and association areas highlights a dynamic adjustment process in the brain, which is crucial for flexible visual understanding and rapid learning when encountering new information.

Disclosures: R. Srinath: None. **M.R. Cohen:** None.

Poster

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AFOSR FA9550-24-1-0235
NIH EY014800
Research to Prevent Blindness

Title: A deep neural network model for identifying LFP oscillatory components underlying phase coding of visual information during eye movements in extrastriate cortex

Authors: *H. AHN[1], G. WENG[2], B. NOUDOOST[3], N. NATEGH[1];
[1]Electrical and Computer Engin., [2]Biomed. Engin., [3]Ophthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: It has been shown that neurons' sensitivity in several brain areas can be represented in the timing of spikes relative to the phase of oscillations in specific frequency bands of the local field potentials (LFP), a phenomenon known as phase coding. However, reading this phase code is challenging due to the fact that LFP signals recorded via microelectrodes reflect a complex mixture of various local and distant oscillatory sources with certain spectrotemporal patterns. Precisely identifying and isolating these oscillatory components is crucial for understanding information encoding at the level of single spikes, which is particularly important for characterizing neurons' time-varying sensitivity during dynamic behaviors such as eye movements. During saccadic eye movements (saccades), the frontal eye field (FEF) sends top-down signals to area V4, modulating visual responses according to eye movement information. Decomposing V4 LFPs to isolate the oscillatory components specifically driven by FEF input is essential for tracing the neural phase code in V4 neurons that represent dynamic sensory information across eye movements. In this study, we introduce an unsupervised learning-based source separation framework to dissect the intricate oscillatory dynamics within the visual cortex, with the goal of decomposing V4 LFP signals into components related to the FEF activity and those intrinsic to area V4. We used simultaneously recorded LFP data from retinotopically corresponding site in FEF (single electrode) and V4 (16-channel linear array electrode) areas in monkeys performing a memory-guided saccade task. This framework was designed to leverage dynamic representation of sensory information at each timepoint relative to saccades, showing the capability of our approach for tracking distinct oscillatory sources with high temporal precision. The characteristics of the isolated FEF-related and V4-intrinsic components across eye movement are assessed by analyzing their time-frequency profiles, spatial distribution within area V4, and their contribution to spike phase locking. The information content of this phase code is evaluated to examine its capacity for decoding visuospatial information across saccades. Our findings provide important insights into the role of brain oscillations in sensory representation at the single-trial level and in the establishment of a precise temporal code enabled by communication between prefrontal and visual areas.

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Poster

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Topic: E.06. Vision

Support: NSF GRFP
NIH EY032900
NIH EY032179

Title: Perisaccadic gating of retinogeniculate transmission in awake macaques

Authors: *R. A. RESSMEYER[1], J. L. YATES[3], G. D. HORWITZ[2];
[1]Bioengineering, [2]Neurobio. and Biophysics, Univ. of Washington, Seattle, WA; [3]Optometry, UC Berkeley, Oakland, CA

Abstract: Saccadic eye movements result in rapid shifts of the retinal image and are accompanied by profound changes in visual perception. One of these changes is saccadic suppression—the observation that detection thresholds for many stimuli increase during saccades. The neural mechanisms of saccadic suppression are poorly understood but may involve gating at the retinogeniculate synapse. To determine whether visual signaling through the lateral geniculate nucleus (LGN) is attenuated during saccades, we recorded LGN spiking activity in awake, behaving macaques using Neuropixels probes.

This first-of-its-kind dataset allowed us to classify LGN units into functional types and, notably, contained pairs of units that may originate from monosynaptically connected retinal ganglion cell (RGC) axons and LGN neurons. This interpretation is strongly supported by converging evidence (Fig. 1a, b): (1) consistent 1-2 ms spike latencies between the two units, (2) identical receptive field properties, (3) distinct spike waveforms, and (4) a dependence of spike transmission probability on the leading unit's inter-spike interval.

Critically, for these pairs of units, the probability of a leading (RGC) spike eliciting a trailing (LGN) spike was dynamically modulated around saccade onset. We observed a decrease in the probability of spike transmission within ~50 ms of saccade initiation, followed by an increase ~70 ms post-saccade (Fig. 1c, d). We then interrogated the putative RGC-to-LGN transfer function using various models to test the hypothesis that perisaccadic LGN activity is explainable from its primary RGC drive alone. Early results suggest that even highly expressive models do not fully account for the observed perisaccadic modulation, suggesting that input from other sources may be necessary.

These results provide insight into how retinal signals are transformed at the first stage of the primate visual pathway during active vision, highlighting dynamic gating of visual signals in the LGN around the time of saccades.

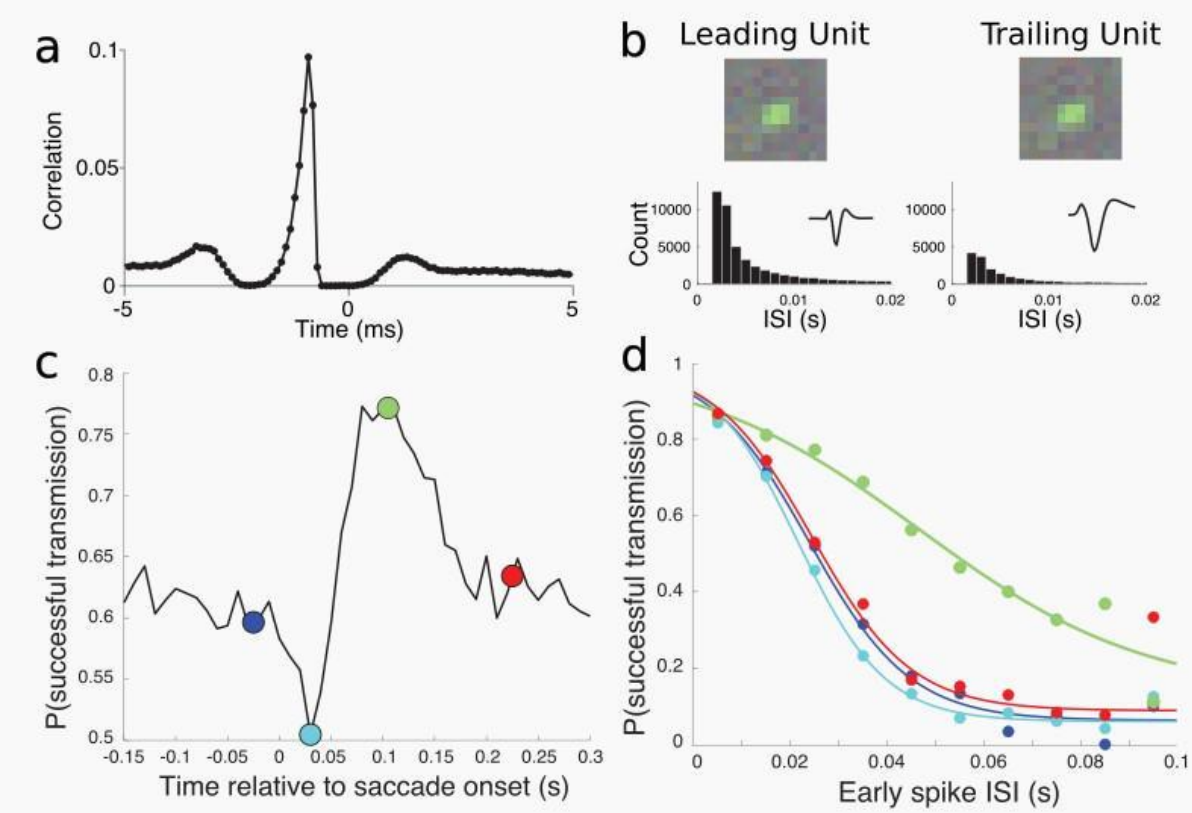

Figure 1. Perisaccadic gating of retinogeniculate transmission. (a) Cross-correlogram between leading and trailing units. (b) Top: spike-triggered averages of the leading (left) and trailing (right) units. Bottom: Interspike interval histograms with spike waveform insets (1-ms duration). (c) Saccade-related changes in RGC-spike transmission probability as a function of time relative to saccade onset. Colored dots represent the middle of the four 50-ms windows that were selected for ISI analysis. (d) Probability of an RGC spike evoking an LGN spike given the ISI between the previous RGC spike. Color corresponds with color in (c). Solid lines depict a logistic regression fit to the data.

Disclosures: R.A. Ressmeyer: None. **J.L. Yates:** None. **G.D. Horwitz:** None.

Poster

PSTR393: Visual System: Responses During Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR393.17/Q16

Topic: E.06. Vision

Support: SNSF 310030_204544
SNSF 31NE30_203974 ERA-net
Carigest Foundation

Title: State dependent target representation in area V4 of macaque visual cortex

Authors: *K. LIZA[1], R. KIENITZ[3], J. CADENA-VALENCIA[1], D. GHEZZI[4], M. C. SCHMID[2];
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Abstract: The precise relationship between cortical activity in the sensory areas and perception remains unclear. Perception of an identical sensory stimulus can differ depending on ongoing neural state. Previous electroencephalogram (EEG) studies in humans have demonstrated differences in the power and phase angle in the theta and alpha frequency bands, particularly in the frontal region, shortly before the stimulus onset between successfully detected (hits) and undetected (miss) trials at the perceptual threshold (Busch et al., 2009; Dugué et al., 2011). In macaque visual cortical area V4, perceptual detection at threshold has been linked to reduced variability in superficial layer and increased synchrony in the cortical layers (Morton et al., 2024). To further characterize neural activity underlying this perceptual variability, we analysed the multi-unit activity (MUA), and local field potential (LFP) recorded from chronically implanted 96-channel Utah arrays in V4 of two macaque monkeys performing a visual detection task. During each trial, a stimulus was presented in the receptive field of V4 neurons followed by a subsequent target flash at variable luminance contrast. The monkeys reported target detection through saccadic eye movements. While stimulus-evoked MUA and LFP responses did not differ significantly between hit and miss trials, we observed an increase in target evoked MUA response during hit trials, despite identical target contrasts. Analysis of variance (ANOVA) confirmed that both behavioural outcome and contrast independently affected the target evoked activity. Hit trials showed fewer micro saccades prior to stimulus or target onset, suggesting enhanced eye position stability may facilitate perceptual performance. Importantly, we also observed a significant increase in beta range power (18-30 Hz) in the pre-target MUA activity after stimulus onset during hit trials, particularly in the superficial layers of V4. This may reflect saccade-related motor preparation activity in V4 preceding the perceptual decision. Together, these results show that a successful perceptual detection is influenced by both behavioural and neurophysiological markers, underscoring the role of area V4 in state-dependent perceptual outcomes.

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Morton, Mitchell P., et al. "Brain state and cortical layer-specific mechanisms underlying perception at threshold." Elife (2024)

Disclosures: K. Liza: None. **R. Kienitz:** None. **J. Cadena-Valencia:** None. **D. Ghezzi:** None. **M.C. Schmid:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Program #/Poster #: PSTR393.18/Web Only

Topic: E.06. Vision

Support: NIH R01 EY031477
AFOSR FA9550-24-1-0235
NIH EY014800
Research to Prevent Blindness

Title: Developing a blind source separation framework for investigating the role of distinct oscillatory sources in creating a phase code of perisaccadic information in area V4

Authors: *A. MAHMOOD ZADEH[1], G. WENG[2], B. NOUDOOST[3], N. NATEGH[4]; [1]Sharif Univ. of Technol., Tehran, Iran, Islamic Republic of; [2]Biomed. Engin., [3]Ophthalmology and Visual Sci., [4]Electrical and Computer Engin., Univ. of Utah, Salt Lake City, UT

Abstract: Understanding how prefrontal inputs influence visual processing during eye movements is key to studying sensory-cognitive integration. The Frontal Eye Field (FEF) has direct projections to visual area V4, and modulates V4 responses and oscillatory power, pointing to the FEF as one source of oscillatory signals in V4. Our goal is to identify specific oscillatory components within local field potentials (LFPs) recorded from V4 that reflect input from the FEF, as well as local oscillatory sources. By isolating these components, we aim to understand how they contribute to phase-based visual encoding, where the timing of neural spikes relative to the phase of ongoing oscillations carries meaningful information, during saccadic eye movements (saccades), which require high temporal precision. To this end, we analyze the LFP and spiking data simultaneously recorded from FEF (single electrode) and V4 (16-channel array) neurons with overlapping receptive fields, in rhesus macaques performing a memory guided saccade task. During this task, a 50 ms visual probe is flashed at one of nine locations around a V4 neuron's receptive field, either during fixation or around saccade onset. The monkey uses a second saccade to report the perceived stimulus location, allowing us to measure perisaccadic alterations in location perception. We isolate the constituent oscillatory components in LFPs using a state-space modeling approach to decompose V4 LFPs into several latent oscillatory components across 4-80 Hz. Using this method, we identify distinct oscillatory components within the V4 LFP that either reflect input from the FEF or arise from local V4 activity. To characterize these sources, we compare the extracted components with those in FEF signals. We also assess the timing of V4 spikes relative to these component oscillations through spike-phase locking and evaluate the capacity of this phase information for decoding stimulus location with high temporal precision across saccades. Moreover, by jointly analyzing their temporal, spectral, and spatial characteristics, we investigate how these components play a role in constructing a dynamic representation of spatial information during saccades. Our findings can provide insight into how sensory information can be reliably read out at the level of single trials- a critical capability underlying our flexible behavior on rapid timescales.

Disclosures: A. Mahmood Zadeh: None. **G. Weng:** None. **B. Noudoost:** None. **N. Nategh:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

Support: NIH/NEI R01EY018839
NIH/NEI R01EY029601
NIH U01 NS131810-01
NIH/ORIP P51 RR000166-46

Title: Dissecting the Visual-Frontal Interactions during Object Recognition in Non-Human Primates

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Abstract: Interactions among brain regions underlie higher cognitive functions such as visual object recognition. We previously reported the representation of occluded figures in areas V4 and prefrontal cortex (PFC) using shape silhouettes. Here, we extended this work by challenging monkeys to recognize animals from impoverished stimuli, a task that requires active figure-ground segregation. We created two types of impoverished 2D stimuli—Mooney and Pointillist stimuli—each containing both the animal figure and a background. In the Mooney stimuli, the figure was generated from shading and silhouette maps derived from a reference 3D object, while the ground was composed of multiple "splat" patches clipped from the same figure. Task difficulty (i.e., ground level) was titrated by the number of surround patches. In the Pointillist stimuli, the figure was defined with dots derived from a reference 3D object mesh with a minimum dot spacing constraint, while the ground was made of dots independently generated using 3D models of bushes. In this case, difficulty was titrated by varying the dot density of the animal figure. In each trial of the recognition task, subjects viewed an impoverished image containing an embedded animal figure for 600 ms, followed by a delay (300 ms), and then made a two-alternative forced choice by touching one of two reference 3D model images. To prevent reliance on low-level feature memorization, in each recording session, 2-3 animals were tested across multiple ground levels, and each animal was presented in various poses. We simultaneously recorded neural activity from V4 and PFC using Neuropixels probes and observed distinct behavioral patterns and neural responses across the two stimulus types. With Mooney stimuli, the subject was able to rapidly and accurately (accuracy = 96% ± 1%, reaction time = 540 ± 51 ms) identify figures regardless of image difficulty, and statistically significant encoding of animal identity regardless of pose or ground noise ($p < 0.01$, t-test or ANOVA) were observed in PFC across 5/8 sessions. While with Pointillist stimuli, task accuracy (73% ± 3%) and reaction time (580 ± 58 ms) changed gradually depending on the image deterioration. In this case, population-level figure signals were absent in the PFC across all sessions (n=3). On the other hand, populations of V4 cells tended to show differential responses across figures with both Mooney (7/8 sessions) and Pointillist stimuli (3/3 sessions). These preliminary results suggest that V4 and PFC may jointly contribute to figure-ground segregation to achieve object

recognition, with V4 more involved in processing visual features and PFC more engaged in higher-level integration.

Disclosures: H. Chen: None. **T. Kim:** None. **A.K. Pasupathy:** None.

Poster

PSTR393: Visual System: Responses During Behavior

Location: SDCC Halls B-H

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Topic: E.06. Vision

Support: NIH R01 EY031477
AFOSR FA9550-24-1-0235
NIH EY014800
Research to Prevent Blindness

Title: Assessing Prefrontal-Visual Communication Across Saccadic Eye Movements

Authors: *A. KHOSRAVIPOUR[1], G. WENG[2], B. NOUDOOST[3], N. NATEGH[4];
[1]Electrical Engin., Sharif Univ. of Technol., Tehran, Iran, Islamic Republic of; [2]Biomed. Engin., [3]Ophthalmology and Visual Sci., [4]Electrical and Computer Engin., Univ. of Utah, Salt Lake City, UT

Abstract: Understanding how the brain dynamically coordinates activity across regions during different phases of visual processing is essential for uncovering the neural basis of spatial perception. In particular, the communication between the Frontal Eye Field (FEF) and visual area V4 plays a critical role during saccadic eye movements, when perception is known to be disrupted, such as in the phenomenon of perisaccadic mislocalization. Building upon a previously published dataset in which rhesus macaque monkeys performed a memory guided saccade task with visual stimulation, we analyze simultaneous local field potential (LFP) recordings from a 16-channel linear array electrode placed in area V4 and one electrode in FEF. Our goal is to examine the directional flow of information between these two regions across time and frequency of LFP oscillations using Granger causality analysis. We divided the data into three distinct temporal windows (fixation, perisaccadic, and memory periods) and performed two complementary analyses. First, we investigated the directionality of FEF-V4 interactions within each individual window, in both the time and frequency domains, to identify whether one region exerted a stronger causal influence over the other during specific task phases. Second, we compared information flow across the three temporal windows to identify meaningful differences in both the magnitude and directionality of LFP-based interactions between FEF and V4 based on task epoch. This window-based analysis enabled us to examine how the dynamics of inter-areal communication evolve in relation to distinct behavioral states, including fixation, saccade execution, and memory retention. Our analysis aims to uncover systematic differences in neural communication between FEF and V4 that may underlie changes in visual perception

associated with saccades. By combining temporal segmentation with directional connectivity measures, this work provides new insight into the functional dynamics of inter-areal signaling and lays the groundwork for future studies linking these dynamics to perisaccadic perceptual phenomena.

Disclosures: A. Khosravipour: None. **G. Weng:** None. **B. Noudoost:** None. **N. Nategh:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Program #/Poster #: PSTR393.21/R2

Topic: E.06. Vision

Support: NIH Grant R01EY022933
NIH Grant R01EY034566
NIH Grant P30EY026877

Title: The contribution of eye movements to retinal and cortical variability in the mouse

Authors: *Y. FARAGALLA[1], J. B. MELANDER[1], J. C. WEDDINGTON[1], Z. ALAOUI[1], D. AU[2], M. D. MENZ[1], S. A. BACCUS[3];
[1]Stanford Univ., Stanford, CA; [2]Neurobio., Stanford Univ., Palo Alto, CA; [3]Neurobio., Stanford Univ., Stanford, CA

Abstract: Natural vision is characterized by frequent eye movements that have received much attention in primates and humans, in which gaze is either controlled by training or measured precisely. Little is known, however, about the influence of eye movements on the visual system of the mouse. Although the current picture of mouse visual cortical responses is that neural responses to repeated stimuli are highly variable, the contribution of eye movements to this variability has not been measured. We presented repeated natural stimuli and white noise in vivo to the mouse and recorded from the mouse primary visual cortex using Neuropixel electrodes to record from populations of neurons. We tracked the eye position and reconstructed the visual stimulus experienced by the mouse during each stimulus trial. We then presented reconstructed visual stimuli containing measured eye movements to the in vitro isolated retina, and recorded retinal ganglion cell activity using a high-density multi-electrode array (MEA). Retinal stimulus presentations included the same natural stimulus sequences with different eye movements, and identical trials that contained the same eye movement trajectories. In this way, we could distinguish variability inherent in the retina from that produced by eye movements. As previously reported, repeated stimuli in vivo that were identical on the video monitor showed higher variability than identical stimuli presented to the retina. However, when stimuli that included the different eye movements for each trial, variability substantially increased, approaching for some cells variability seen in cortical neurons. We further captured cortical responses to natural scenes with convolutional neural network models, and found that the ability

to predict cortical firing increased when the image was corrected for the actual gaze measurements on each trial. Although it was not possible to measure cortical responses to repeated stimuli in the absence of eye movements, our results suggest that a substantial fraction of inferred cortical variability is not neural, but due to eye movements that differ across trials. These findings indicate that active sensation substantially influences mouse visual responses, and highlight the importance of incorporating measurements of eye movements in studies of the mouse visual neural code.

Disclosures: Y. Faragalla: None. **J.B. Melander:** None. **J.C. Weddington:** None. **Z. Alaoui:** None. **D. Au:** None. **M.D. Menz:** None. **S.A. Baccus:** None.

Poster

PSTR393: Visual System: Responses During Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR393.22/R3

Topic: E.06. Vision

Support: Hessian Ministry of Higher Education, Research, Science and the Arts, the Excellence Program "The Adaptive Mind"
SFB/TRR 135
ERC-2023-STG-101117441

Title: Prior scene context shapes neural dynamics of face detection

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Abstract: Humans can detect faces within a fraction of a second-faster than any other object category. This remarkable speed raises a key question: How can top-down signals influence such a rapid perceptual process? While recent findings show that prior scene context facilitates face detection, the neural mechanisms underlying this effect remain unclear. Here, we used electroencephalography (EEG) to investigate how prior scene context shapes the temporal dynamics of face detection in naturalistic settings. Participants (N=44) viewed real-world scenes, each containing a single face located either on the left or right. Each scene was preceded by either a faceless version of the same scene (preview condition) or a gray screen (no-preview condition). Participants performed a face detection task, with 10% of trials containing foil images without faces. Using multivariate pattern analysis, we decoded face location (left vs. right) from both time-locked broadband EEG and time-frequency-resolved signals. Broadband decoding revealed enhanced face-location information in the preview condition between 171-291 ms after stimulus onset, and in the no-preview condition at later stages between 392-1039 ms. This pattern suggests that prior context facilitates early spatial processing, while its absence leads to delayed, compensatory processing. Time-frequency decoding showed higher decoding

accuracy in the preview condition in both the alpha band (8-13 Hz; 322-553 ms) and beta band (14-30 Hz; 397-492 ms), suggesting top-down orienting of spatial attention. Together, these results suggest that prior scene information shapes face detection via distinct neural signatures across time and frequency domains. When a preview is available, the brain rapidly encodes face location-likely leveraging a prior representation of the scene's spatial layout-followed by low-frequency activity associated with spatial orienting. Without a preview, enhanced face location information emerges later, consistent with bottom-up sensory integration. Our findings highlight how top-down contextual information and bottom-up sensory input are dynamically integrated in the brain, even during rapid visual processing.

Disclosures: S. Tasliyurt Celebi: None. **D. Kaiser:** None. **K. Dobs:** None.

Poster

PSTR393: Visual System: Responses During Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR393.23/R4

Topic: E.06. Vision

Support: R01 Grant

Title: Neurodynamics of Active Face Emotion Perception with Eye Tracking and Intracranial EEG

Authors: *C. BECKER[1], M. RICHEY[1], J. A. GONZÁLEZ-MARTÍNEZ[1], T. J. ABEL[2], W. J. LIPSKI[1], A. S. GHUMAN[1];
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Abstract: Other people's faces capture our attention in a distinctive way: we make rapid eye movements both toward faces that appear in our periphery and across facial features, engaging specialised neural systems as we do so. These gaze patterns are unique and systematic, and disruptions in them are a hallmark of face perception deficits. And yet, much of what we know about the neuroscience of face perception relies on paradigms where faces are presented abruptly at central fixation, overlooking the dynamic role of eye movements in natural vision. Here, we characterize the neurodynamics of face perception during unrestrained sequential fixations around a face using eye tracking and intracranial EEG in human participants. Participants free viewed static images of happy, angry, fearful, and neutral facial expressions for 1500 milliseconds. Behaviorally we found an initial freezing of eye movements upon stimulus onset, followed by a first saccade around 200ms consistently across participants. This timing aligns with the N170 timeframe and give rise to the question of how much of early face-related potentials are associated with this oculomotor freezing and the planning of the first saccade. Comparing responses across multiple fixations (fixation-related potentials) to traditional event-related potentials (ERPs) elicited by sudden onset faces uncovered large differences in the neurodynamics between the onset response and neural responses to subsequent fixations. These

neurodynamics highlight the neural foundations of the dynamic active sensing process in face perception during free viewing.

Disclosures: C. Becker: None. **J.A. González-Martínez:** None. **T.J. Abel:** None. **W.J. Lipski:** None. **A.S. Ghuman:** None.

Poster

PSTR393: Visual System: Responses During Behavior

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Topic: E.06. Vision

Support: NIH R01EY032162
NIH/ NEI 5 T35 EY007149-24
AAO Ezell Fellowship/ Cushing Foundation (NR)

Title: Atypical nerve routing and impaired early visual functioning do not diminish higher visual functioning in people with albinism

Authors: *J. SKERSWETAT[1], N. ROSS[2], O. WYNN[2], C. IDMAN-RAIT[2], P. BEX[3]; [1]Ophthalmology and Vision Sci., Univ. of California, Irvine, Irvine, CA; [2]New England Col. of Optometry, Boston, MA; [3]Psychology, Northeastern Univ., Boston, MA

Abstract: Albinism is characterized by misrouting of the nerves at the optic chiasm and structural changes in striate cortex organization, leading to well-documented impairments in early visual functions, e.g., reduced acuity and contrast sensitivity. However, the extent to which higher-level visual processes, such as object discrimination, are affected remains unknown. To address this gap, this study developed the novel method Foraging Interactive D' -FInD Object to investigate object discrimination capabilities in people with albinism (PwA). FInD Object is a self-administered, adaptive paradigm, which displays six charts, each containing a 3*3 grid of object image pairs taken from the THINGS database (Hebart et. al., 2019). The image pairs were selected from four object categories (Animals, Plants, Body parts, E-Devices) from the 27 THINGS categories, and differed by adaptively-selected semantic distances. The participants' task was to click on pairs where objects were different things. A d' sensitivity function followed by a decision function were used to estimate object discrimination thresholds. First, repeatability of FInD Object was investigated in 24 normally sighted adults, then 18 PwA (mean VA: 0.43 logMAR) completed the task and were compared to 14 fully corrected controls (0.08logMAR). Bland Altman analysis showed no significant mean biases for thresholds for any object category, but significant positive proportional biases for all categories($p<0.01$), & coefficients of repeatability range of 0.24-Plants to 0.72-E-Devices. A repeated measures ANOVA showed no significant differences in object semantic discrimination between PwA and controls, and no significant interaction. The adaptive, self-administered FInD Object task generated generally repeatable results, but unevenly distributed object discrimination thresholds that may be

improved with additional instruction or training charts. Remarkably, we found that despite diminished visual acuity in PwA, this did not significantly affect their ability to discriminate between object semantics compared to controls. These results suggest that object processing is intact in PwA.

Disclosures: J. Skerswetat: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); JS is founder of and owns equity in PerZeption Inc.;FInD (Foraging Interactive D prime) is co-invented by JS, patented (pending) and owned by Northeastern University, Boston & licensed to PerZeption. **N. Ross:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NCR has received funding for research from Eschenbach Optik, however this does not pertain to the contents of this abstract. **O. Wynn:** None. **C. Idman-Rait:** None. **P. Bex:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PB is founder of and owns equity in PerZeption Inc.;FInD (Foraging Interactive D prime) is co-invented by PJB, patented (pending) and owned by Northeastern University, Boston & licensed to PerZeption.

Poster

PSTR393: Visual System: Responses During Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR393.25/R6

Topic: E.06. Vision

Support: NIH EY028811-01
NSF IIS-2143440

Title: A Meta-Analysis of 59 Studies Reveals Factors Driving Choice Probability Magnitude

Authors: *A. PLETENEV[1], C. C. PACK[2], G. C. DEANGELIS[1], R. M. HAEFNER[1];
[1]Univ. of Rochester, Rochester, NY; [2]Neurol & Neurosurg, McGill Univ., Montreal, QC, Canada

Abstract: Choice probability (CP), a measure of the correlation between a single neuron's activity and the subject's decision, is considered a key quantity for understanding the neural basis of perception (Parker & Newsome, 1998). Although CP values above chance have been widely reported, their magnitude varies substantially across studies. We conducted a meta-analysis of 59 studies published from 1996 to 2024, extracting 153 mean CP values (typically one per monkey) and associated experimental parameters, to investigate what drives CP magnitude.
Surprisingly, we observed a decline in reported CPs over time, independent of recording technique, reminiscent of the "decline effect" often seen for effect sizes across scientific fields ($p < 0.001$).
Less surprisingly, we found a modest positive relationship between mean CP and neuronal sensitivity to the task, measured as the psychometric-to-neurometric threshold ratio ($p = 0.003$).

Much of the variability in sensitivity was explained by how well stimuli were tailored to the neurons' tuning properties. Interestingly, the across-study slope appears smaller than within-study slopes. Notably, the intercept of the CP-sensitivity relationship was significantly above 0.5, suggesting a suboptimal read-out, or a choice-related signal not perfectly aligned with neural sensitivity for the task (0.525, $p < 0.001$).
Regression analyses revealed that mean CP also varied with brain area, stimulus duration, and task type. V1 showed lower CP than downstream areas, but beyond that CP values did not appear to depend on an area's position in the cortical hierarchy. Also, longer stimuli were associated with higher CP, consistent with a top-down feedback contribution ($p = 0.001$). Finally, while CPs in fine discrimination tasks were lower than in coarse discrimination tasks, this effect was entirely explainable by reduced neuronal sensitivity raising the question of why neurons are found to be less sensitive (relative to behavior) for fine discrimination tasks. Detection tasks showed higher mean CPs than coarse discrimination tasks, but this effect can again be explained by differences in neural sensitivity. Only bistable stimuli yielded significantly higher CP even after controlling for other factors, likely reflecting stronger top-down modulation or collective dynamics ($p = 0.002$).
By identifying key experimental factors that systematically shape CP, our findings provide empirical constraints for competing theoretical accounts and guide future investigations into choice-related signals in particular, and the neural basis of perception in general.

Disclosures: A. Pletenev: None. **C.C. Pack:** None. **G.C. DeAngelis:** None. **R.M. Haefner:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.01/R7

Topic: E.07. Visual Sensory-Motor Processing

Support: CIHR Grant 165823

Title: Gaba regulation of proprioception and locomotion in the spinal cord

Authors: *N. DE SILVA[1], K. HARI[3], D. J. BENNETT[2];
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Abstract: Proprioceptive feedback is essential for coordinating and guiding movement, yet the spinal mechanisms regulating this process remain incompletely understood. Previous work by Hari et al. demonstrated that GABAA receptors at axon branch points reduce spike propagation failure via primary afferent depolarization (PAD). This mechanism enhances conduction of sensory information. Specifically, PAD is regulated by a trisynaptic circuit involving GAD2 neurons that release GABA onto the receptors, V3 glutamatergic neurons that activate the GAD2

neurons, and motor neurons that receive direct input from sensory afferents. We further investigated the role of nodal GABAA signaling using transgenic mice with targeted disruptions to this circuit, assessing their performance on a ladder walking task. This task effectively isolates proprioceptive function, as mice rely on sensory feedback rather than vision to guide limb placement. Wild-type (WT) mice exhibited approximately 20% foot fault error, whereas models with impaired GABAA signaling showed error rates approaching 50%, despite preserved motor abilities. Conversely, mice with enhanced nodal GABA release demonstrated superior accuracy, highlighting the importance of GABA in proprioceptive transmission. To assess this in the context of spinal cord injury (SCI), we performed T8/9 hemisections in various cohorts. Mice with disrupted GABAA signaling displayed impaired ipsilateral stepping and reduced foot placement precision. Interestingly, they also exhibited improved proprioception in the contralateral limb, prompting further investigation into this paradoxical effect. We then used L655708 to pharmacologically block GABAA receptors which impaired sensory guided stepping in WT intact mice, but restored unskilled locomotion in T8/9 transects. This reflects a reduction in sensory input and an increase in motor output. Data was quantified via foot fault rates and BMS scores ($p < 0.05$). These findings show the dual and context dependent role of nodal GABAA signaling in regulating proprioceptive and motor function which should be considered in therapeutic intervention.

Disclosures: N. De Silva: None. **K. Hari:** None. **D.J. Bennett:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

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Program #/Poster #: PSTR394.02/R8

Topic: E.07. Visual Sensory-Motor Processing

Title: Impact of eye movements and orofacial movements on mouse visual cortex

Authors: *A. SYEDA[1,2], M. A. NUÑEZ[3], L. ZHONG[3], M. PACHITARIU[4], C. STRINGER[5]; [1]Janelia research campus, Arlington, VA; [2]Johns Hopkins Univ., Baltimore, MD; [3]Janelia Res. Campus, Ashburn, VA; [4]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA; [5]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Previous studies have found that the mouse primary visual cortex (V1) is correlated to various orofacial movements. However, recent work in primates suggests that monkey V1 is primarily modulated by eye movements, not orofacial movements (Talluri et al 2023). In mice, it remains unclear how much eye movements contribute to the modulation of neural responses in the presence or absence of visual input compared to other orofacial movements. To determine the contribution of eye movements to mouse V1 activity, we recorded the activity of thousands of V1 neurons using two-photon calcium imaging while presenting a visual stimulus or in darkness and monitoring eye movements and orofacial behaviors with a camera. We utilized Facemap, our AI-based framework, to precisely track orofacial movements and predict neural

activity from mouse behavioral videos. Facemap uses a convolutional neural network to track keypoints on the mouse face and obtain precise movement information. In addition, Facemap uses a deep neural network model with temporal convolutions to capture movements at various timescales for neural activity prediction, outperforming previous linear models. With this model, we found that eye movements influence a small fraction of activity in the mouse visual cortex compared to other orofacial behaviors. In the presence of visual input, eye position predicts ~10% variance explained after accounting for retinal input whereas orofacial behaviors account for 20-35% of variance explained. We next investigated the relationship between orofacial movements and eye movements. We found increased whisker and nose movements around the time of saccades but no difference in running. We developed a convolutional neural network to predict eye movements from whisker and nose movements, and vice versa. We found whisking and sniffing behaviors have more information about eye movements than vice versa. These results suggest that orofacial movement signals play a larger role in visual cortical processing in mice compared to primates.

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Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.03/R9

Topic: E.07. Visual Sensory-Motor Processing

Support: NIH NINDS R35
SCGB Pilot Award

Title: Circuit mechanisms for binocular distance estimation in flies

Authors: *D. S. MEARNS, **I. SHIN**, M. ARAGON, Y. PARK, H. SEUNG, M. MURTHY; Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Many animals use information from the two eyes to measure distance to objects in the world, and while multiple mechanisms have been proposed, to date no circuit has been mapped at synaptic resolution that implements such a computation. Some insects have fixed-range stereopsis, whereby binocular cues are used to determine whether an object is at a specific distance from the animal, which differs from proposed models in mammals where the distance to objects in the binocular visual field are encoded in a disparity map. In *Drosophila*, while binocular integration is thought to be important for many behaviors, there is limited evidence for behaviors that involve binocular distance estimation. Courtship is an ethologically relevant behavior where male flies pattern their chasing and singing behavior relative to female distance. How males determine their distance to females is not known. Here, we identify a putative circuit for fixed-range stereopsis in *Drosophila*. LC14 neurons bilaterally innervate the optic lobes and

were previously postulated as a substrate for binocular distance computations. Connectome analysis reveals three subtypes of LC14, and we identify sexually dimorphic connectivity in LC14a1, which may therefore play a role in sex-specific behaviors such as courtship. Using the connectome, we predict that the receptive field of LC14a1 is matched to a conspecific in the center of the visual field ~4 mm away, a distance at which male flies undergo a state switch between near- and long-range courtship behaviors. We also identify specialized circuitry in the center of the visual field where LC14a1 imparts binocular cues to a subset of neurons previously implicated in courtship. Whole brain 2-photon calcium imaging in awake, behaving male flies presented with females confirmed our predictions from the connectome that the binocular zone selectively responds to females at close range in the binocular visual field, and that binocular responses propagate to downstream brain regions consistent with connectome predictions. Finally, silencing LC14a1 neurons disrupts the switch between near- and long-range courtship behaviors. Together, we have identified a potential circuit mechanism for fixed-range stereopsis in insects that gates an ethologically relevant behavior.

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Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

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Topic: E.07. Visual Sensory-Motor Processing

Support: Wellcome trust
Simons foundation
Fundação para a Ciência e Tecnologia
Champalimaud Foundation

Title: Comparing the activity of four neuromodulators during decision-making

Authors: K. BOUGROVA[1], M. CARANDINI[2], G. A. CHAPUIS[3], D. CROMBIE[1], E. E. DEWITT[1], M. FAULKNER[2], L. FREITAS-SILVA[1], ***C. QUADRADO**[2], G. RAISER[1], O. WINTER[1], I. BRAIN LAB[3], Z. F. MAINEN[1];
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Abstract: The four principal neuromodulators (NMs) are thought to play critical roles in decision-making by implementing the computational substrates of flexible decision-making, such as reward prediction error. While, dopamine (DA)-mediated reward prediction errors are well-studied, other neuromodulatory systems - serotonin (5-HT), norepinephrine (NE), and acetylcholine (ACh) - have been shown to encode prediction errors, uncertainty, and surprise. This illustrates how there is a lack of understanding of how these NM contribute individually and

operate collectively to support flexible behavior, which highlights the challenges posed by studies that often target a single NM in different decision-making paradigms, preventing direct comparison.
To address this, we leveraged the framework of the International Brain Laboratory (IBL) to survey these NMs in a highly standardized decision-making task shown to generate reproducible behavioral and neurophysiological results. We used fiber photometry to measure bulk Ca2+ activity of genetically-targeted neuromodulatory populations expressing the GCaMP6f calcium indicator. Here, we present preliminary findings from 5 core NM nuclei across ~750,000 trials in ~40 double-transgenic mice performing a visually guided decision-making task, during initial learning up to proficient task performance.
Consistent with the existing literature, DA activity diverges based on choice outcomes - increasing its activity following rewards and decreasing after errors - with graded amplitude changes depending on the trial difficulty. Meanwhile, 5-HT shows a strong response to incorrect choices, consistent with an unsigned prediction error. Interestingly, serotonergic activity was suppressed prior to stimulus onset, suggesting a possible role in maintaining focus and task-related behavioral states. NE activity is highly variable both within and between mice, suggesting a possibility of specialized roles for distinct sub-populations. Lastly, ACh exhibits greater amplitude of activity at early training stages, specially in correct trials, demonstrating a possible role in learning.
These results underscore the distinct yet complementary roles of NMs in signaling reward-related contingencies that could guide future behaviors in a decision-making context. The integration of fiber photometry data with IBL's behavioral and brain-wide electrophysiological datasets provides a powerful tool to explore the interactions of the four NMs in learning and decision-making.

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Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

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Program #/Poster #: PSTR394.05/R11

Topic: E.07. Visual Sensory-Motor Processing

Title: Development of the perception-action loop during natural hunting in ferrets from eye opening

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Abstract: Critical periods represent windows of heightened plasticity in neural development, yet traditional models view experience as a feedforward modifier of passively received sensory input. In reality, animals actively shape their experience through evolving behavior, especially during early life when sensorimotor systems co-develop. Here, we present a new experimental platform to study the emergence of visually-guided natural behavior from the moment of eye opening in ferrets—a species with a primate-like visual system and rapid developmental timeline. We evoke instinctive prey pursuit using a fictive hunting paradigm and track behavioral development using markerless 3D motion capture (FreeMoCap) and head-mounted eye tracking (Pupil Labs). This setup allows day-by-day quantification of full-body kinematics, gaze patterns, and head-centered optic flow during ethologically relevant tasks. We observe a rapid transformation in gaze stabilization, trajectory planning, and coordination of body and eye movements over the first two weeks following eye opening, marking the transition from disorganized locomotion to precision targeting and successful pursuit. These measurements provide a high-resolution map of how motor competence and visual sampling co-evolve during the early critical period, offering a behavior-first framework for defining developmental milestones. By capturing the reciprocal dynamics between visual input and emerging action, this work lays a foundation for future studies linking natural behavior to cortical circuit development and provides a blueprint for modeling early sensorimotor learning in AI systems.

Disclosures: E. Dale: None. **P. Queen:** None. **A. Cherian:** None. **J.S. Matthis:** None. **B. Scholl:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

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Program #/Poster #: PSTR394.06/R12

Topic: E.07. Visual Sensory-Motor Processing

Support: BRAIN Initiative RF1NS128895

Title: Neural mechanisms of visually driven action selection in larval zebrafish

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Abstract: To successfully navigate natural visual environments, the vertebrate brain must selectively process the most relevant sensory cues. Visual processing and action selection in mammals involve cortical and subcortical brain-scale networks. However, the simultaneous recording of multi-region, single-cell level neural circuit activity and behaviors poses a significant challenge to our understanding of these processes. Here, we investigated the brain-scale neural circuits underlying visually driven action selection in the optically and genetically

accessible larval zebrafish. In the wild, fish must hunt for resources and escape predators while stabilizing their body position to counteract being swept away by water currents, also known as optomotor response (OMR), a visually evoked behavior. To investigate how larval zebrafish perform this action selection, we performed closed-loop high-speed behavioral tracking with simultaneously presenting moving gratings and dot stimuli, simulating global motion cues induced by water flow and moving prey. By comprehensively characterizing visually evoked orientation changes, swim bout dynamics, and eye vergences, we demonstrate that stereotypical hunting behaviors dominate ongoing OMR-associated behaviors, significantly reducing the animal's response to global visual motion cues. This action selection bias towards hunting behaviors is the strongest when the dot moves across the binocular visual field. This strong suppression of OMR by the prey-like stimuli is consistent across different motion directions of gratings and prey-like stimuli, and remains robust regardless of preceding behavior. Nevertheless, the strength of the behavioral suppression is modulated by competing stimulus salience, including dot size, grating speed, and spatial frequency. With simultaneous 2-photon calcium imaging and behavioral tracking in head-embedded zebrafish, we show that local prey-like stimuli modulate neural activity in known visual neural circuits, including the retinorecipient optic tectum (OT), pretectum (PT), and anterior hindbrain (aHB). Utilizing optogenetic photostimulation of dot-responsive OT neurons, we generated functional interaction maps between OT and PT. Our results show that multiple visual processing brain areas interact to process visual signals and guide appropriate action selection in vertebrates navigating complex sensory environments. More generally, we demonstrate a multiregional neural architecture where information from the whole field environment and local objects are parallelly represented and selectively processed for context-dependent action selection.

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Poster

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Topic: E.07. Visual Sensory-Motor Processing

Support: The Smith-Kettlewell Eye Research Institute
NIDILRR RERC Program grant 90REGE001

Title: Effect of visual task demands on head stabilization in older adults with and without central visual field loss

Authors: *C. P. AGATHOS[1], A. VELISAR[2], N. M. SHANIDZE[3];
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Abstract: Age-related macular degeneration is the leading cause of visual impairment in older age, damaging the central retina. In AMD, unstable eccentric fixation is commonly observed when employing a peripheral retinal locus, instead of the fovea and may combine with age-related vestibular deficits, thus affecting gaze (eyes + head) stabilization. This can alter body movement patterns and increase fall risk. Head stabilization while moving is critical to maintain a stable reference for the visual, vestibular, and auditory systems, and to better control gaze. There is evidence of functional, active gaze tasks like visual search improving postural and locomotor stability in healthy young adults. These adaptations are possible thanks to the tight link between the postural and gaze systems. It is possible that head stabilization improves when gaze has a more active, and potentially functional role. Should this hold in those with AMD, new training protocols can be developed to improve both oculomotor control and head stabilization, facilitating daily life tasks. Participants included 5 visually healthy older adults (2F, age range: 60-78), one older adult with monocular AMD, (M, 82) and one with binocular AMD (F, 84). They all stepped in place (SIP) with and without performing a visual search task, while their eye and head movements were tracked with an IMU and eye-tracker, respectively. Targets and distractors were projected on a screen (58°×48°) at 2m and participants reported the number of targets found during successive trials (Xs among other letters, between 4 and 9 per trial). The visual search task reduced head motion in several ways, suggesting better stabilization. We found a significant reduction in anteroposterior head acceleration and acceleration variability in all participants, with a greater reduction in those with AMD. A similar trend was found for vertical head acceleration metrics ($p=0.07$). In addition, we saw a significant reduction in angular velocity in roll during the search task in all participants. Data collection and analysis are ongoing. Findings suggest preserved sensorimotor adaptation abilities in older adults with and without CFL, exhibiting postural adaptation to achieve the supra-postural, visual task. Should we observe consistent results in a larger sample, these outcomes offer promising avenues for rehabilitation and training. How eye movements are used during visual search will also be important to consider to better understand the relationship between the visual, oculomotor and postural components of this task.

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Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

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R21AG081998 (PI: Erin Kinnally)

Title: Age and sex differences in social content preferences in rhesus macaques (Macaca mulatta)

Authors: *A. CUNNINGHAM[1], L. CENICEROS[2], R. SUBEDI[2], A. BROWN[2], A. CRUZ[2], E. L. KINNALLY[3];
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Abstract: As people age and social networks dwindle, there is potential for depressive affect and behaviors to increase. With an increase in virtual communication and media consumption, it is becoming a concern that the potential for social isolation and depressive feelings are also increasing. Additionally, the conversation surrounding negativity in media and its cyclical exchange with people's wellbeing grows as more time is spent engaging with electronic entertainment. Research suggests that quantifying what type of content an animal prefers to watch may reflect their affective state. For instance, it has been found that rhesus monkeys who have recently experienced a stressor will spend more time looking at negative social imagery. Previous analysis from this study has shown that what we expect of older animals - to show more depressive behaviors - is actually the opposite of what is happening - older animals display less depressive behaviors. The goal of this study is to examine social content preferences in aging animals, and relate these preferences to well-being. 37 animals aging from 15 to 29 years old, were assessed via Geriatric Biobehavioral Assessment at CNPRC. Animals were presented with short affiliative (prosocial) and aggressive social videos of other rhesus macaques. Their gaze data was recorded using Mangold EyeTech software on an EyeOn eye-tracker. Across the entire cohort of animals, there was no significant overall difference in how much animals directed gaze toward affiliative vs. aggressive video content. However, content preference differed by age. Middle aged animals (15, roughly equivalent to 60 human years) spending the most time watching the negative social video, and older animals (19-29 years of age, roughly equivalent to 80-110 human years) spending more time watching the affiliative video ($p < 0.05$). There is also a significant sex effect, with older males looking more at positive social stimuli and older females looking more at the negative content ($p < 0.05$). These preliminary findings point to age and sex differences in what type of social stimuli they prefer to watch. This may support previous findings that aging animals may actually be experiencing less negative affect, and therefore possibly have a better well being, as they age than previously expected. Future studies will examine the relationship of content preference with affective and cognitive status in aging.

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Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

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Program #/Poster #: PSTR394.09/R15

Topic: E.07. Visual Sensory-Motor Processing

Support: PRIN 2022 Prot. 2022 MRWN2K

Title: Exploring drone-based cinematography: a high-density EEG study

Authors: *M. P. SOMMA[1], M. CALBI[1], N. LANGIULLI[1], G. D'ADAMO[1], A. KOLESNIKOV[1], M. UMILTA[2];
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Abstract: Recent research suggests that neural mechanisms supporting action observation extend beyond real-life interactions to mediated experiences, including those evoked by cinematic techniques such as camera movements. Notably, Heimann and colleagues (2014; 2019), investigated the impact of different camera movements on sensorimotor system activation, finding that Steadicam-filmed scenes elicited a stronger Event-Related Desynchronization (ERD) of the mu rhythm, thus reflecting a sensorimotor resonance mechanism activation. More recently, Kolesnikov and colleagues (2025) further explored this phenomenon using as stimuli videos depicting a staircase filmed with a drone-mounted camera, varying in Drone Movement Direction and Human Presence. Participants evaluated each video in terms of perceived movement, physical and emotional involvement and liking. Their findings confirmed that drone cinematography can evoke embodied and emotional responses, supporting the role of embodied simulation in cinematic experience. Building on this evidence, we developed a novel experimental paradigm to explore whether drone-mounted cameras, which resemble "flying Steadicams", can elicit sensorimotor resonance. High-density EEG was recorded from 28 naïve individuals (with no prior piloting experience) and 15 expert drone pilots (ongoing recruitment) while viewing 3-second clips of an empty staircase filmed by a drone under three conditions: Ascending, Descending and Still. In the Action Observation condition, participants passively viewed the clips, while in the Action Execution condition, they were asked to indicate, by pressing a button, whether the last seen video depicted a movement. In the naïve group, time-frequency analysis revealed a nonspecific ERD in the alpha and beta frequency bands, possibly reflecting distinct visuospatial processing of the scenes. Although data collection for the expert group is ongoing, we hypothesize that drone pilots will exhibit a more pronounced and spatially specific ERD over central scalp regions corresponding to the hand motor representation, consistent with sensorimotor activation related to their motor expertise in drone piloting. This effect is expected to emerge particularly during the observation of ascending and descending movements, reflecting enhanced sensorimotor resonance driven by motor familiarity and embodied motor representations developed through drone piloting. These findings will contribute to the growing literature on the neural underpinnings of cinematic experiences and will offer new insights into how drone-based camera movements engage the viewers' sensorimotor system.

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Poster

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Program #/Poster #: PSTR394.10/R16

Topic: E.07. Visual Sensory-Motor Processing

Title: Analysis and identification of visual-motor integration in human motor control

Authors: *A. KILIC[1,2], O. KARAGOZ[2], M. M. ANKARALI[3];
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Abstract: This research focuses on the complexities of visual-motor integration in humans, using target-tracking tasks to emphasize the dynamics of human-in-loop systems. Drawing from the foundations of the pursuit and compensatory control models, the study accentuates the profound influence of feedback mechanisms and the predictability of inputs on performance outcomes.
The research design included four distinct target-tracking tasks, each examining different aspects of visual-motor integration. These tasks were characterized by variations in trajectory types, including single-frequency sinusoids and sum-of-sine, and differences in experimental scenes, ranging from visibility of the target and operator to only error information representation. We collected position and velocity data from 25 participants using a haptic device.
An accurate response was observed when participants received direct visual feedback, even under unpredictable stimuli. Another observation was the overall better tracking performance when the input was predictable compared to unpredictable stimuli. Also, we compared the performance enhancement created by the input and feedback. We saw that increasing the predictability of the input was more effective than increasing the feedback supplied to improve the performance. Moreover, the consistent emergence of a "U" shaped magnitude response across unpredictable input experiments alludes to potential intrinsic properties of the human motor system, possibly influenced by factors such as neural resonance.
This research integrates conventional methodologies with more realistic scenarios, offering a layered and more nuanced understanding of human capabilities and limitations in motor control models and opening up opportunities for designing systems that can better interact with or augment human abilities.

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Poster

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Program #/Poster #: PSTR394.11/S1

Topic: E.07. Visual Sensory-Motor Processing

Title: Spontaneous Task-Driven Emergence of Dorsal and Ventral Stream Analogues in Neural Networks: Correlations with Human EEG

Authors: *T. REZA[1], E. JORDAN[1], K. PATEL[1], J. TANG[1], H. DONNELLY[1], M. ASKARI[1], M. NIEMEIER[2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Univ. Toronto, Etobicoke, ON, Canada

Abstract: Visual information is processed in the brain through two anatomically and functionally distinct pathways: the dorsal stream, which supports visually guided actions such as grasping, and the ventral stream, which supports visual recognition tasks such as object classification. A longstanding hypothesis suggests that these streams arise from different computational goals and optimization pressures during development. Supporting this view, artificial neural networks (ANNs) trained on classification tasks exhibit different representational properties from those trained for action-oriented tasks such as grasp detection. However, prior comparisons across models have been limited by differences in architecture and training objectives, making it difficult to isolate the role of task-driven optimization. To address this, we designed a unified map-based ANN trained simultaneously on object classification and grasp planning using a shared double-log loss function that is agnostic to task type. This multi-task learning framework allowed both functional outputs to emerge from a common feature extractor. To quantify task-specificity in network representations, we used Neuron Shapley, a game-theoretic method for estimating each unit's marginal contribution to task performance. We observed that early convolutional layers showed strong overlap in importance scores across tasks, while deeper layers exhibited increasing divergence, indicating the development of task-specialized representations. To assess biological relevance, we performed representational similarity analysis (RSA) comparing ANN activations with EEG data recorded from human participants engaged in analogous grasping and recognition tasks. EEG patterns during recognition aligned more closely with the ANN's classification output, while grasping-related EEG responses were more similar to the grasp output. Additionally, we conducted connectivity analysis using a graph neural network framework on ANN activations and found a separation in wiring patterns within the shared layers, consistent with emergent stream differentiation. These findings suggest that task-driven optimization in a unified ANN may support the spontaneous emergence of dorsal and ventral stream analogues, with representational and functional patterns that align with human EEG responses.

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Poster

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Program #/Poster #: PSTR394.12/S2

Topic: E.07. Visual Sensory-Motor Processing

Support: Danna and Albert R. Broccoli Charitable Foundation
HINRI Labs/ Ross Mason
Pat and Nancy McKey

Title: Transcutaneous Spinal Neuromodulation Combined with Task-Specific Training Restores Sensorimotor and Autonomic Function After 12 Years of Clinically Complete SCI

Authors: *I. MONTOYA[1], C. JOHNSON[2], H. ZHONG[2], H. R. TORRES SOLANO[2], J. RICHTER[2], P. LOOMBA[2], V. EDGERTON[3,2];
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Abstract: The adult human spinal cord retains a robust, underutilized capacity for functional reorganization, even in individuals with clinically complete injuries. We report longitudinal outcomes from a 34-year-old male with chronic (>12 years) traumatic T4-T5 AIS A SCI and right C5-C7 brachial plexus root avulsion. Over five years, the participant engaged in a high-frequency protocol combining noninvasive transcutaneous spinal cord stimulation (tSCS) with task-specific, high-repetition, constraint-induced activity-based training. Interventions included exoskeleton-assisted overground walking, therapist-facilitated treadmill stepping, sit-to-stand transitions, and recumbent elliptical movement—all delivered under neuromodulatory input. Progressive reductions in robotic assistance (100% to 55%) coincided with volitional modulation of locomotor output, enhanced lower-limb joint kinematics, and electromyographic activity consistent with re-engagement of descending supraspinal pathways. Load-dependent and proprioceptive feedback below the lesion re-emerged, enabling upright ambulation without visual compensation, despite complete right upper-limb deafferentation. Structural adaptations included hypertrophy of paralyzed musculature and bone mineral density gains approaching normative z-scores.
Autonomic recovery was substantial. Bladder capacity increased from 50 to 230 mL with return of urge awareness and improved detrusor regulation. Bowel care time was halved, and rectal sensation returned. Thermoregulatory capacity normalized. Sexual function improved markedly, with restored genital sensation, ejaculatory reflex, and increased intercourse frequency, accompanied by improved sperm count and motility. Cardiovascular autonomic control stabilized, with normalized orthostatic tolerance, blood pressure, and favorable heart rate variability.
This case demonstrates that targeted spinal neuromodulation, when combined with precision rehabilitation, can re-enable coordinated motor and autonomic functions long after the chronic phase of SCI. These findings challenge fixed definitions of injury completeness and expand the window for meaningful recovery.

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Poster

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Program #/Poster #: PSTR394.13/S3

Topic: E.07. Visual Sensory-Motor Processing

Support: Danna and Albert R. Broccoli Charitable Foundation, Nanette and Burt Forester.

Title: The Use of Low-Amplitude Transcutaneous Spinal Neuromodulation to Improve Functional Mobility, Balance, and Autonomic Function in Children with Spina Bifida: A Case Series

Authors: S. HIGGINS[1], C. JOHNSON[2], H. ZHONG[2], *V. EDGERTON[3,2], S. HASTINGS[4,2]; [1]All About Kids Threapy Services, Lawrenceville, GA; [2]Rancho Res. Inst., Downey, CA; [3]Univ. of Southern California, Keck Sch. of Medicine, Neurorestoration Ctr., Los Angeles, CA; [4]Susan Hastings Pediatric Physical Therapy, San Jose, CA

Abstract: Children with spina bifida (SB) present with significant motor, balance, and autonomic impairments secondary to their neurologic spinal lesion. Emerging evidence supports transcutaneous spinal neuromodulation (TSN) as a promising adjunctive intervention to enhance sensory-motor and autonomic outcomes after spinal cord lesions. Our goal is to evaluate the effects of subthreshold stimulation amplitude (mA) TSN +ABT on functional outcomes noted above in SB patients with emphasis on the ambulatory performance of a child born with a sacral myelomeningocele. Six children, 23 mo-13 years, were treated. Pre- and post-intervention outcome measures included an average of 11.3% increase in balance assessments. All six participants also demonstrated clinically meaningful improvements in one or more functional domains. Subjects with more consistent focus on postural alignment combined with TSN attained the greatest improvements. Notably, a 4½-year-old child who received the most consistent TSN with ABT over an 8 week program, 14 sessions incorporating TSN/24 total sessions demonstrated substantial ambulatory gains, increasing from 2- 28 fully independent steps with barefoot and from 4 - 84 meters when wearing her knee, ankle, foot orthosis (or KAFOs), while postural alignment improved from 37° to just 1° forward trunk lean with slow, controlled walking and to 15° with fast walking. Balance test scores improved by 13.5% and functional test by 16% for standing and walking skills, far exceeding the minimal detectable change of 7%. Sensory changes were noted after 3 weeks with expressions of itchy legs and feet "feeling funny" when stepping on the treadmill, as well as inconsistently identifying when bowel movements had occurred. These data are consistent with 5 other cases of patients having been treated less frequently and consistently. Another participant demonstrated improvements in bowel and bladder awareness and continence starting 1 month post TSN during toileting and 4 months post TSN during ABT, suggesting potential autonomic benefits. TSN +ABT demonstrated positive trends in balance, motor control, and autonomic functions in children with SB. These case series observations provide strong evidence that this intervention can facilitate correction of multiple physiological disorders particularly in younger patients by engaging subject specific activity interventions.

Disclosures: S. Higgins: None. **C. Johnson:** None. **H. Zhong:** None. **V. Edgerton:** None. **S. Hastings:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.14/S4

Topic: E.07. Visual Sensory-Motor Processing

Support: NSF Grant 2050833

Title: Traveling waves link sensorimotor areas during visually-guided action

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Abstract: Human actions are often guided by visual input, yet how this information is communicated from visual to motor cortices to generate action remains unclear. Our recent study showed that when visual working memory (WM) content was used to guide action, feedforward theta waves(2-6Hz) propagated from task-relevant visual areas to motor regions, followed by beta waves(14-32Hz) in the opposite direction. These waves predicted response initiation and cessation, respectively, indicating that feedforward and feedback traveling waves may underlie the mechanism initiating and controlling WM-guided behavior. Here, we asked whether this phenomenon generalizes to actions guided directly by visual input. Participants performed a visually guided response task adapted from the previous WM study, in which they viewed two bars, both on the left and right sides of the screen, and each with a specific orientation. A cue then indicated which bar to report, and participants responded by adjusting a response bar using the hand on the same side as the tilt direction of the cued bar. Stimulus location and response hand were fully crossed across trials, allowing us to assess the direction and timing of communication between sensory and motor areas during visually guided action. We collected and analyzed two datasets (N=24 and N=10), which differed in whether the stimulus remained visible during the response phase—one with the stimulus present and one without. In both, we observed the same feedforward theta and feedback beta waves in the same temporal order as in the WM-guided task. Additionally, we observed a novel alpha-band traveling wave(8-12Hz) that emerged specifically during response execution - a pattern absent in the previous WM task. While the functional role of the alpha wave remains to be determined, our results suggest that traveling waves support actions guided by visual cortical input, whether maintained in memory or directly perceived.

Disclosures: C. Luo: None. **T. Houghton:** None. **E.F. Ester:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

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Program #/Poster #: PSTR394.15/S5

Topic: E.07. Visual Sensory-Motor Processing

Support: Danna and Albert R. Broccoli Charitable Foundation, Nanette and Burt Forester

Title: Transcutaneous Spinal Neuromodulation Combined with Activity-Based Therapy Leads to Rapid Improvements in Functional Vision in Children with Cerebral Palsy and Cortical Visual Impairments

Authors: P. STAMPE[1], C. JOHNSON[2], H. ZHONG[2], V. EDGERTON[3,2], *S. HASTINGS[4,2]; [1]Pia Stampe CP Consulting, Eureka, CA; [2]Rancho Res. Inst., Downey, CA; [3]Dept of Neurolog. Surgery and Neurorestoration Ctr., Univ. of Southern California, Keck Sch. of Medicine, Neurorestoration Ctr., Los Angeles, CA; [4]Susan Hastings Pediatric Physical Therapy, San Jose, CA

Abstract: Children with cerebral palsy (CP) often exhibit a range of motor, balance and sensory impairments. Cortical Visual Impairment (CVI), which affects approximately 60-70% of children with CP, significantly hinders motor development, learning, and executive function. Many infants with severe CP initially respond only to light and may later progress to recognizing simple black, white, or red images.Transcutaneous spinal neuromodulation (TSN), when paired with activity-based therapy (ABT), is emerging as a promising intervention aimed at enhancingsensorimotor integration and skill acquisition. The objective of this work is to describe the rapid and constant changes from daily TSN+ABT on functional vision in three children with CP,ages 2 to 13 years. All three children, demonstrated notable improvements in functional vision within 2-3 months of initiating TSN+ABT. Gains included enhanced visual attention, object and facial recognition, spatial localization, and environmental awareness.Notably, there were slight differences between children with CP and their responses to TSN+ABT. Child #1, diagnosed with severe CVI including visual ataxia, began TSN+ABT at 28 months of age. Within three months, the child demonstrated the ability to perceive multiple objects on a surface, shift visual attention between items, and recognize two-dimensional images, as well as people and objects at a distance.Over the course of treatment, the child progressed from GMFCS V to IV, eventually leading to independently navigating a wheelchair in complex visual settings at 3.5 years of age. Child #2, age 13 with a diagnosis of periventricular leukomalacia and spastic quadriplegiaand GMFCS IV, demonstrated within a month of TSN+ABT, noticed and inquired about visual details in familiar environments. By the third month,the child was able to identify visual features in dynamic settings, including reading street signs and billboards from a moving vehicle. Child #3 began daily TSN+ABT at 15 months of age. Within four months, the child demonstrated visual responses to illuminated objects. By 20 months, visual tracking was evident; by 22 months, the child could recognize familiar faces; and by 27 months, the ability to interpret and imitate facial expressions had emerged.These preliminary findings suggest that TSN combined with ABT may facilitate rapid improvements in functional vision in children with CP, including those with severe CVI. Further investigation is needed to validate these results and elucidate the underlying mechanisms.

Disclosures: P. Stampe: None. **C. Johnson:** None. **H. Zhong:** None. **V. Edgerton:** None. **S. Hastings:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.16/S6

Topic: E.07. Visual Sensory-Motor Processing

Support: NTT-OIST collaboration Fund
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JP23H04978

Title: Diverse Cortical Dynamics Underlying Body Motion in Freely Moving Marmosets

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Abstract: Sensorimotor systems interacting with complex environments rely not only on the canonical perception-decision-action pathway, but also on latent processes, including reflexive mechanisms from visual motion to arm movements, which would be critical for reaching adjustment in unstable postural conditions (Gomi 2008 Curr. Opin. Neurobiol.).While these processes are separately embedded in different neural mechanisms, they function in a coordinated fashion. To identify distinct information processing modes in the cortex, we recorded cortical-wide electrocorticograms (ECoG) from a freely behaving marmoset, in which electrodes cover widespread cortical areas from frontal to occipital regions. Head motion was monitored via a head-mounted inertial measurement unit (IMU), and behavior was recorded via multiple synchronized external cameras. Analysis of an 18-minute segment from one individual using cross-correlation, PCA, and Temporal Autoencoders for Causal Inference (TACI; Calderon & Berman 2024), revealed multiple cortical activity patterns associated with posture and movement. One pattern exhibited widespread cortical activation time-locked to movement in the low-frequency band (1-30 Hz). This frequency-band activity rose in the prefrontal and motor areas prior to movement onset (~-50 msec), then propagated to parietal and occipital regions (~90 msec). Another pattern was observed in a limited set of frontal, temporal, and parietal channels, where gamma-band activity (80-200 Hz) increased during movement. Causality analysis revealed that the activity in this limited set of channels improved the predictability of movement signals, indicating a predictive component of motor-related processing. However, these localized signals were not consistently aligned with head-motion; in some cases, strong gamma activity occurred with minimal motion and vice versa. This suggests that these signals may not merely reflect dynamical aspects of movement but could be associated with the processing related to the changes in visual input, or eye or segmental body movement. Together, our findings demonstrate that distinct ECoG signal patterns related to spontaneous movements can be identified in the cortex of freely moving marmosets. Low-frequency signals may reflect top-down voluntary control mechanisms, while localized gamma activity, commonly found in a subset of ECoG channels including visual cortex, may be involved in predictive processing related to movements.

Disclosures: Y. Yamane: None. **M. Komatsu:** None. **K. Doya:** None. **H. Gomi:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.17/S7

Topic: E.07. Visual Sensory-Motor Processing

Support: SNSF Ambizione

Title: A novel task for the implicit measurement of visuomotor integration and sense of agency

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Abstract: Motor control requires the real-time integration of sensory information with motor-efference copies. To accomplish this, visual and proprioceptive information needs to be integrated in a precise and selective manner. Not only is the effective integration necessary for accurate closed-loop motor control, but it is also hypothesized to be fundamental to our subjective experience of causing our actions, our sense of agency. Continuous comparison of intentions and outcomes is required to accurately assess self-causation. While functional connectivity has been proposed as a mechanism for the weighting of visuomotor feedback, systematic investigation of this hypothesis remains limited.
Current research on agency and sensorimotor integration in humans tends to rely on self-reporting, which might present a skewed view, as reported experience is an indirect measure of neural processes and their associated behavior. Moreover, tasks typically involve discrete movements, which are less ecological and constrain the investigation of functional connectivity. In order to mitigate the limits of discrete movements and subjective reporting, we present a novel task that implicitly measures visuomotor integration during continuous movement and links it to the subjective sense of agency. Participants are asked to produce specific motor behaviors by controlling a cursor. Noise is introduced to modulate the integration of visual information. Additionally, visual feedback is distorted to subconsciously prompt an adaptation in motor behavior. The extent of behavioral adaptation is taken as a proxy of the integration of visual information. We modelled our task within a Bayesian framework, inferring self-causation probability from incongruency, in order to gauge the strength of the visuomotor feedback. Preliminary results validate the correction for the distortion as an implicit measure of visual information integration and sense of agency. Moreover, the results indicate Bayes-like behavior in terms of the weight assigned to the visual feedback. These results are well explained by the Bayesian model.
The task we present enables us to implicitly measure the integration of visual information and agency for precise motor behavior. Paired with electrophysiological recordings, this task will allow the investigation of functional connectivity dynamics and possibly elucidate the employed neural computations. Furthermore, being completely implicit and requiring little cognitive control, the task can be readily translated to animal models.

Disclosures: J.S. Nieuwenhuis: None. **S. Micera:** None. **T. Bertoni:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.18/S8

Topic: E.07. Visual Sensory-Motor Processing

Title: Respiration as a means to facilitate perception

Authors: *N. CHALAS[1], E. BALESTRIERI[2], O. ABBASI[2], J. GROSS[2], D. KLUGER[2]; [1]Institute For Biomagnetism and Biosignal Analysis, Münster, Germany; [2]Univ. of Muenster, Muenster, Germany

Abstract: Recent research increasingly recognizes the coupling between bodily rhythms—like respiration—and neural oscillations, with respiration-modulated brain oscillations (RMBOs) shaping action, perception, and cognition. In a visual near-threshold perception task using magnetoencephalography (N = 30), we demonstrate that perceptual sensitivity is heightened when stimuli are presented during the inspiration phase, coinciding with increased cortical excitability. Participants adaptively adjusted their breathing patterns to task demands, with stronger adjustments linked to better performance, highlighting the voluntary and predictive nature of this body-brain coupling. Pupil dynamics, a proxy for noradrenaline-driven arousal, were influenced by spatial cues, with larger pupil diameters during cued trials predicting improved performance. Neural analyses revealed that both alpha-band activity (linked to visual perception) and beta-band activity (linked to motor preparation) were modulated by respiration phase and cue predictability. Multivariate source-localized and Granger causality analyses further showed that respiration phase modulates information flow within the RMBO network, directly influencing perceptual sensitivity. Collectively, these results underscore respiration as a flexible, behaviorally relevant modulator of neural dynamics, facilitating sensory sampling with nested body-brain dynamics.

Disclosures: N. Chalas: None. **E. Balestrieri:** None. **O. Abbasi:** None. **J. Gross:** None. **D. Kluger:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR394.19/S9

Topic: E.02. Somatosensation – Touch

Support: NIH NINDS BRAIN U19 NS137920

Title: Motor patterns and neuronal circuits in control of head movements with breathing in rodents

Authors: *S.-M. LIAO[1], B. FRIEDMAN[2], M. MORENO[1], J. TAKATOH[4], F. WANG[5,6], D. KLEINFELD[1,3];
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Abstract: Rodents interact with their environment using head and orofacial motor actions to actively move sensors through their peripersonal space. More so, the rhythmic components of motion of the vibrissae, tongue, jaw, nose, and the head are coordinated by the respiratory cycle as the rodents explore or forage (Kurnikova, Moore et al., 2017; Liao & Kleinfeld, 2023). A hierarchical structure for the whisking oscillator circuit, where the breathing center (preBötC) resets the rhythmicity of an all-inhibitory whisking oscillator (vIRT) in the brainstem, has been experimentally determined (Moore, Deschênes et al., 2013; Deschênes et al., 2016; Takatoh, Prevosto et al., 2022) and theoretically modeled (Golomb et al., 2022). Nonetheless, how the circuits for other orofacial motor actions, notably the head oscillators that are coordinated by breathing to form output patterns that can change with behavioral states, await comprehensive investigation.

In this study, we delineate the motor and premotor circuits underlying head control in mice by leveraging retrograde monosynaptic tracing techniques (Takatoh, Park, Lu et al., 2021). We first map the motor neuron pools of dorsal and ventral neck muscles in the caudal brainstem and the cervical cord with cholera toxin subunit B. We thereby obtain reconstructed 3-D distributions of labeled cells innervating sternomastoid and biventer cervicis, two neck muscles that exhibit distinct phase relationships with sniffing. Our preliminary data show that biventer cervicis, but not sternomastoid, is innervated by bilateral motor neurons, distantly spanning from the caudal brainstem to caudal cervical cord. These data motivate further study on the neural pathways of bilateral neck muscle co-activation and coordination in head control.

We aim to completely unravel the premotor circuitry, identifying putative neck oscillators and their pathways of receiving respiratory signals. We inject AAVretro-Cre into selected neck muscles in mice, followed by a second injection into the upstream motor neuron pools using Cre-dependent helper AAVs that co-express the avian virus receptor (TVA) and the coat glycoprotein, and a third injection into the same motor pool using EnvA-pseudotyped G-deleted rabies. In view of the observation that neck motor neurons form an elongated distribution along the spinal column, we test the delivery of viruses via the intracisternal and intramuscular routes to improve labeling efficiency. To promote data sharing, we upload our data to BrainSharer (brainsharer.org) for collaborative annotation and analysis.

Disclosures: S. Liao: None. **B. Friedman:** None. **M. Moreno:** None. **J. Takatoh:** None. **F. Wang:** None. **D. Kleinfeld:** None.

Poster

PSTR394: Sensorimotor Transformation: Behavior and Whole Animal

Location: SDCC Halls B-H

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Program #/Poster #: PSTR394.20/S10

Topic: E.02. Somatosensation – Touch

Support: NIH Grant U19NS137920

Title: Distinct brainstem premotor circuits control different aspects of rhythmic licking

Authors: J. TAKATOH[1], ***I. BANDO**[2], P. THOMPSON[2], V. PREVOSTO[2], C. ULDRY LAVERGNE[2], A. HARRAHILL[3], F. WANG[2];
[1]Stony Brook Univ., Long Island, NY; [2]MIT, Cambridge, MA; [3]Univ. of Utah Sch. of Med., Salt Lake City, UT

Abstract: Many orofacial movements such as breathing, whisking, licking, and chewing are rhythmic. However, the neuronal identities of oscillators that generate rhythmic licking and chewing remain largely unknown. *Phox2b*$^+$ neurons in the intermediate reticular nucleus (IRt) and the parvocellular reticular formation (PCRt) have been implicated in generating rhythmic licking. Moreover, our previous three-step monosynaptic rabies virus tracing revealed that IRt and PCRt contain premotor neurons innervating hypoglossal (XII) motoneurons involved in licking. We hypothesize that *Phox2b*$^+$ premotor neurons in IRt and/or PCRt are crucial in rhythmogenesis for licking behaviors. We have successfully labeled *Phox2b*$^+$ premotor neurons in the IRt and PCRt, IRt_{Phox2b}→XII and $PCRt_{Phox2b}$→XII, using a viral-genetic intersectional strategy. We discovered that chemogenetic silencing of IRt_{Phox2b}→XII resulted in reduced licking amplitude, while rhythmic tongue movement was minimally affected. In contrast, chemogenetic silencing of $PCRt_{Phox2b}$→XII resulted in single licks with long intervals, showing drastically diminished rhythmicity. These findings indicate that these two regions may modulate distinct aspects of tongue movement. Supporting this idea, continuous laser stimulation of IRt_{Phox2b}→XII neurons elicited prolonged tongue protrusions with inconsistent rhythmicity across trials. Activating $PCRt_{Phox2b}$→XII neurons elicited stereotyped rhythmic licking for the duration of the stimulus. These findings suggest that IRt_{Phox2b}→XII neurons control amplitude and initiation of licking but are insufficient to maintain licking rhythm, whereas $PCRt_{Phox2b}$→XII neurons are necessary and sufficient for generating licking rhythm. Together, our data support a model in which distinct premotor subcircuits contribute differentially to the patterning and execution of rhythmic licking.

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Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.01/Web Only

Topic: E.08. Multisensory Integration

Support: NIH R01NS121040

Title: Regional Characterization of Hemodynamic Response Function in White Matter in Human cerebral cortex

Authors: *J. CUSTARD[1], A. VINOGRADOV[2], C. SCOPICE[1], N. J. FESHARAKI[2], J. KIM[2]; [1]UTHealth Sci. Ctr. McGovern Med. Sch., Houston, TX; [2]Neurosurg., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Functional magnetic resonance imaging (fMRI) studies have traditionally focused on measuring blood oxygen level dependent (BOLD) signals in gray matter (GM), often overlooking signals in white matter (WM). In this study, we examined WM hemodynamic response functions (HRFs) across multiple cortical regions using fMRI at 3T. We defined a self-reciprocal depth coordinate, the normalized distance w, which is zero at the GM-WM interface and reaches unity at the pial surface. By extending each trajectory from the GM-WM interface into the WM, we identified WM regions associated with adjacent GM. We then analyzed HRFs within the WM in the w range of -0.2 to -0.6 to minimize artifacts and undesirable noise in deep WM, and to avoid partial volume effects from adjacent GM regions. Despite differences in absolute amplitude between GM and WM, peak amplitudes of the positive HRFs (pHRFs) in WM showed strong correlations with those in GM across all three subjects. We also found moderate to strong correlations in the initial negative response (INR) of the negative HRFs (nHRFs) between GM and WM. HRFs in both GM and WM exhibited similar temporal dynamics for both pHRF and nHRF. Overall, we found reliable and significant HRFs in WM. These moderate to strong correlations in amplitude and temporal similarity between WM HRF and GM HRF suggest that the WM HRF is both reliable and functionally relevant, and is closely associated with the GM HRF.

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Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.02/S11

Topic: E.08. Multisensory Integration

Title: The Impact of Spatialized Audiovisual Cues on Incidental Motor Sequence Learning in an Immersive 3D CAVE Environment

Authors: *S. GOYAL[1], M. R. WATSON[4], D. A. TOVAR[2], M. T. WALLACE[3];
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Abstract: This study investigated the impact of spatialized audiovisual cues on incidental motor skill acquisition within an immersive 3D CAVE environment using a serial reaction time task (SRTT). Stimuli were presented in fixed spatial and temporal 12-element sequences across four conditions: Spatialized Audiovisual (C1), Non-spatialized Audiovisual (C2), Audio Only (C3), and Visual Only (C4). At the final learning block (Block 14), RTs were: C1: 450ms, C4: 490ms, C2: 540ms, and C3: 680ms. This demonstrated that the Spatialized Audiovisual condition (C1) achieved the lowest RTs, highlighting the efficiency benefits of integrated spatialized cues and the facilitative effect of spatial audio (C1 < C2). Preliminary data showed consistent learning across all conditions, with reduced response times (RTs) over the experiment. A Linear Mixed-Effects Model (LMM) analysis of learning trends (rate of RT improvement) revealed a significant Condition x BlockCount_numeric interaction ($p<.0001$). The Visual Only condition (C4) exhibited the steepest learning curve (estimated trend = -0.01706), demonstrating significantly faster learning than all other conditions (vs. C1: $p<.0001$; vs. C2: $p<.0001$; vs. C3: $p=.0001$). Conversely, the Non-spatialized Audiovisual condition (C2) showed the slowest learning rate (estimated trend = -0.00355), significantly slower than C1 ($p<.0001$), C3 ($p<.0001$), and C4 ($p<.0001$). Sequence-specific learning was confirmed by a randomized probe block, causing a substantial RT increase from pre-probe levels ($p<.001$). However, a one-way ANOVA on probe costs revealed no significant effect of Condition ($p>0.05$), suggesting similar performance disruptions. Our cutting-edge 3D CAVE enabled unprecedented manipulation of spatialized audio. The differential learning rates highlight the nuanced impact of sensory cues. This study emphasizes the significant facilitative effect of spatialized auditory cues on motor skill acquisition, suggesting that rich, spatially accurate auditory information, particularly when integrated with visual input, profoundly enhances complex motor sequence learning efficiency. It offers critical insights into neurocognitive mechanisms underlying multisensory integration and human performance in immersive environments.

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Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

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Program #/Poster #: PSTR395.03/S12

Topic: E.08. Multisensory Integration

Title: A artificial neural tactile sensing system

Authors: *D. BAE[1], H. KIM[1], K. BAK[1], S. BAE[1], P. SUNGBAE[1], H. JUNG[2], Y.-J. KIM[2], S. CHUN[1];

[1]Korea Univ., Sejong, Korea, Republic of; [2]Med. Device Develop. Ctr., Cheongju, Korea, Republic of

Abstract: Humans detect tactile stimuli through a combination of static pressure and dynamic vibration signals using different types of cutaneous mechanoreceptors. The artificial tactile sensing and perception system is of interest in the development of robotics and prosthetics, and artificial receptors. However, constructing systems with human-like capabilities remains challenging. Here, we propose an artificial neural tactile skin system that mimic the human tactile recognition process using a multimodal tactile sensor and a signal-converting system. The CNT-PDMS composite tactile sensors were designed to collect static pressure data like slow adaptive (SA) mechanoreceptors, while the fast adaptive (FA)-mimicking PVDF-TrFE film sensors were used to detect dynamic vibration. The sensors respond to pressure and vibration selectively, similarly to SA and FA mechanoreceptors in human skin. The signal-converting system was conducted using neural spike trains that were obtained by recorded response patterns of mouse nerves ex vivo to pressure and vibration stimuli to the hindpaw. We show in an ex vivo test that undistorted transmission of the output signals through an afferent tactile mouse nerve fibre is possible, and in an in vivo test that the signals can stimulate a rat motor nerve to induce the contraction of a hindlimb muscle. We also use our tactile sensing system to develop an artificial finger that can learn to classify fine and complex textures by integrating the sensor signals with a deep learning technique. The approach can also be used to predict unknown textures on the basis of the trained model.

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Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

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Program #/Poster #: PSTR395.04/T1

Topic: E.08. Multisensory Integration

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Tab Williams Foundation

Title: Benchmarking Multisensory Integration: Reliability and Superadditivity in Animals and Humans

Authors: D. A. PORADA, B. E. STEIN, ***B. A. ROWLAND**;
Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The integration of information across different sensory modalities is a fundamental neural process that underlies normal perception and behavior. Studies of the phenomenon in humans often report substantial variation in the magnitude of the impact of multisensory

integration on different behavioral outcomes. To establish a baseline estimate of the inter- and intra-subject reliability of multisensory integration, as well as the scale of its effect on perception and behavior, we first conducted a comprehensive retrospective analysis of seven animal studies spanning 35 years using a benchmark visual-auditory detection and localization task. This extensive dataset enabled a robust assessment of the consistency and magnitude of multisensory integration while accounting for common sources of experimental variability. It revealed that multisensory behavioral performance was consistently above the most stringent referent criterion, the probabilistic sum of the unisensory performance levels. This "superadditive" effect was further consistent with the predictions of a biologically informed model of optimal information synthesis in which visual and auditory input signals are summed before being nonlinearly transformed into observable behaviors by a decision-making process. Using these findings, we developed a virtual reality analog of this task for humans in which a dynamic algorithm was used to calibrate task-relevant information during testing. With this calibration, the measured magnitude of multisensory enhancement was quantitatively similar to that obtained in animal studies. Test results were consistent within the same participant across multiple testing sessions and comparable across participants. The task was optimized to require less than <35 minutes for participants to complete, suggesting that this paradigm may provide a simple approach for quantifying multisensory integration in different contexts and an effective means of evaluating multisensory dysfunction and rehabilitation in clinical populations.

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Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.05/T2

Topic: E.08. Multisensory Integration

Title: Neuronal evidence for dynamic filtering in distance estimation

Authors: *C. ZHANG[1], Y. GU[2];
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Abstract: Numerous psychophysical or behavioral studies have shown that self-motion signals are critical for path integration-based navigation in environments without clear landmark cues, for example, vestibular or optic flow could be deployed by the brain to estimate the traveled distance through a temporal-accumulation process. However, the underlying neural mechanisms are largely unclear. In the current study, we trained two macaques to perform a self-motion distance discrimination task, in which they judged whether their traveled distance was shorter or longer than an internal, referenced distance. Self-motion was simulated using optic flow and inertial linear translation, presented either separately as unimodal conditions, or in combination, which were all interleaved in one experimental session. After training, the animals discriminated

traveled distance well on each cue, and showed improved performance during cue-combined condition in a way consistent with Bayesian optimal prediction. We then recorded neuronal activity in the posterior parietal cortex including LIP, VIP, 7a and MST while the animals performed the task. Population decoding analyses revealed that LIP and VIP encoded more information about traveled distance than MST and 7a. To explore the computational strategy underlying distance estimation, we introduced modified trapezoidal motion profiles with asymmetric acceleration and deceleration phases. Behaviorally, monkeys overestimated distance in high-acceleration conditions compared to low-acceleration ones, suggesting using a dynamic filtering strategy. Sensory input is also less weighted during steady-state motion, probably to reduce the accumulated error over time. Both tuning strength and population decoding accuracy were reduced during the deceleration phase, supporting the dynamic filtering hypothesis. An artificial recurrent neural network (RNN) exhibited similar dynamic filtering behavior at both the behavioral and representational levels. Thus, our results indicated that the dynamic filtering could serve as a general and efficient computational strategy for optimal estimation of traveled distance from self-motion cues.

Disclosures: C. Zhang: None. **Y. Gu:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.06/T3

Topic: E.08. Multisensory Integration

Title: Faster Together: How Timing and Meaning Shape Audiovisual Integration

Authors: *H. M. MARSHALL[1], M. T. WALLACE[2];
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Abstract: The brain's ability to integrate information across sensory modalities enables faster and more efficient responses to behaviorally relevant stimuli. However, the extent to which this multisensory benefit depends on the temporal alignment and congruency of incoming semantic signals remains understudied. In this study, we investigated how sensory modality and stimulus onset asynchrony (SOA) shape response speed to semantic stimuli. 10 participants completed an audiovisual color discrimination task consisting of seven blocks, each corresponding to a different SOA ranging from -50.07 ms (auditory first) to +50.07 ms (visual first). Within each block, participants completed 64 randomized trials evenly divided across four conditions: auditory-only (A), visual-only (V), audiovisual congruent (AVC), and audiovisual incongruent (AVI). On audiovisual trials, the visual and auditory stimuli were offset according to the block's SOA. Participants responded via keypress to indicate the perceived color. Response times (RTs) were calculated relative to the onset of the first stimulus. After removing implausible RTs (<50 ms or >2000 ms) and incorrect responses, repeated-measures ANOVAs were conducted at each SOA to assess the effect of modality on RT. Across all offsets, significant main effects of

modality were observed. Pairwise comparisons revealed multisensory facilitation in AVC trials, with significantly faster RTs than both A and V trials across most SOAs, and race model violations at -50.01, -16.67, 0, and +16.67 ms offsets. Moreover, there were no significant differences in AVC RTs across SOAs. The persistence of RT benefits at near-synchronous offsets suggests that the brain flexibly integrates signals within a narrow temporal window. In contrast, AVI trials, where the auditory and visual stimuli conveyed conflicting semantic information, elicited slower and more variable responses, with no consistent RT effects relative to unisensory conditions. These findings provide clear behavioral evidence that audiovisual congruency and temporal alignment jointly modulate response speed. The presence of multisensory facilitation in congruent trials, but not incongruent ones, highlights the role of semantic consistency in perceptual integration.

Disclosures: H.M. Marshall: None. **M.T. Wallace:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.07/T4

Topic: E.08. Multisensory Integration

Support: TÜBİTAK Grant 123E155

Title: A Low-Latency, Modular Closed-Loop Platform for Cross-Species Multisensory Experiments

Authors: *A. DEMIREL[1], O. KARAGOZ[3], I. UYANIK[2];
[2]Electrical and Electronics Engin., [1]Hacettepe Univ., Ankara, Turkey; [3]Middle East Tech. Univ., Ankara, Turkey

Abstract: Precise, closed-loop control of multimodal stimuli is essential for dissecting how brains fuse information across the senses. We introduce a fully modular experimental platform that delivers visual, mechanosensory, and electrosensory cues in real time to diverse aquatic model organisms—including *Eigenmannia virescens* (weakly electric fish) and *Danio rerio*(zebrafish). The hardware core consists of high-precision linear stages and rotary actuators that position stimulus objects along user-defined trajectories with <0.2 mm spatial error. Animal and stimulus positions are captured at 25 fps by an infrared-illuminated camera and processed by GPU-accelerated tracking on an NVIDIA Jetson system. Latency between an animal's movement and the consequent stimulus update is <10 ms, enabling feedback gains high enough for naturalistic sensorimotor coupling. Interchangeable, 3-D-printed modules snap onto the motion stages to tailor the sensory channel: (i) LED arrays or patterned cylinders for visual paradigms, (ii) transparent mounts carrying conductive plates for electrosensory studies, and (iii) a brushless-driven flow tunnel for mechanosensory rheotaxis tests. A Robot Operating System (ROS) backbone links tracking, control, and actuator nodes, allowing rapid prototyping of

behavioral controllers via Python or C++. One-button calibration routines align camera and actuator coordinate frames across experimental arenas of 5-50 cm. Benchmark experiments reproduced classic refuge-tracking in *E. virescens* and optomotor responses in *D. rerio* with closed-loop gains indistinguishable from published values, validating both temporal fidelity and spatial accuracy. The open, reconfigurable design lets investigators swap species or stimulus modalities within minutes, making the system a versatile tool for studying multisensory integration, active sensing, and motor control. Ongoing extensions integrate multichannel electrophysiology and deep-learning-based pose tracking to further broaden experimental reach.

Disclosures: A. Demirel: None. **O. Karagoz:** None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.08/T5

Topic: E.08. Multisensory Integration

Title: Bayesian Causal Inference as a unifying account of a variety of multisensory temporal tasks

Authors: *H. ZHU[1], L. SHAMS[2];
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Abstract: Background: In multisensory research, three behavioural paradigms dominate attempts to gauge audiovisual integration: illusion tasks (e.g., the sound-induced flash illusion, SiFI), simultaneity judgements (SJ), and temporal-order judgements (TOJ). Across all three, investigators typically compute a temporal-binding window (TBW)—the span of asynchronies still perceived as a single event—and treat its width as a descriptive hard sensory threshold for integration. Aim: We present a mechanistic account showing that TBW width in three classic paradigms is an outcome of a unified Bayesian Causal Inference (BCI) process on multidimensional similarity, and not a hard threshold for integration. Methods and Results: Experiment 1 used a 2-dimensional sound-induced-flash-illusion (SiFI) task in which auditory-visual numerosity and relative onset times of the flashes and beeps were independently varied (n = 24). A four-parameter BCI model captured both flash and beep reports with high fidelity (median $R^2 = 0.93$) and outperformed forced-fusion and maximum-likelihood models ($\Delta BIC > 80$). Crucially, integration remained robust only when the audiovisual signals were concordant on both dimensions—numerosity and onset time. A salient discrepancy in either cue degraded the evidence for a common source and precipitated a steep drop in fusion probability. Therefore, the temporal-binding "window" is not a fixed timing threshold; it stretches or contracts in proportion to the total cross-modal congruence, reflecting Bayesian weighting of multidimensional similarity. In Experiment 2 we analysed simultaneity-judgment (SJ) and temporal-order-judgment (TOJ) data collected in a previous study (n = 19). Extending BCI with a decisional bound, a single parameter set per observer reproduced both SJ and TOJ

psychometrics (median $R^2 = 0.87$). The same BCI model could account for human data in all three tasks; and the resultant TBW in each task. Conclusion: Altogether, these results show that the width of the temporal-binding window is not a primitive sensory limit but a consequence of Bayesian causal inference applied to noisy, multidimensional sensory input. By providing a single quantitative framework that explains illusion data as well as simultaneity- and order-judgment behaviour, this framework reconciles findings that have often been treated separately.

Disclosures: H. zhu: None. **L. Shams:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.09/T6

Topic: E.08. Multisensory Integration

Support: TUBITAK Grant No:123E155

Title: A dual-refuge closed-loop behavioral system for investigating multisensory integration in weakly electric fish

Authors: *O. KARAGOZ[1], D. UÇAR[3], A. KOLAYLI[2], M. M. ANKARALI[1], I. UYANIK[4]; [1]Electrical and Electronics Engin., [2]Biol., Middle East Tech. Univ., Ankara, Turkey; [3]Biol., [4]Electrical and Electronics Engin., Hacettepe Univ., Ankara, Turkey

Abstract: Multisensory integration enables animals to interpret complex environments by combining sensory inputs dynamically. However, the neural mechanisms underlying how animals weight multiple sensory modalities in real-time behavior remain elusive, partly due to methodological challenges in independently manipulating sensory inputs during natural behavior. To address this, we developed a novel dual-refuge closed-loop behavioral system for the weakly electric fish, *Eigenmannia virescens*, to independently control visual and electrosensory inputs during a natural refuge-tracking task. The system comprises an outer agarose refuge, visually distinct yet electrically transparent, and an inner transparent plexiglass refuge, providing robust electrosensory cues without visual contrast. Both refuges are motorized independently, allowing us to create congruent or conflicting sensory conditions in real-time. Using this setup, we systematically varied sensory reliability by adjusting environmental lighting intensity using LEDs and applying band-limited electrical noise at varying intensities to the water. Fish reliably adjusted their refuge-tracking strategies, dynamically shifting reliance from visual to electrosensory cues as sensory conditions changed. To quantitatively understand this behavior, we developed a dynamic state estimation model capturing multisensory integration processes. This model accurately predicted observed behavioral adjustments across various sensory conditions, highlighting the adaptive weighting of sensory inputs. Our integrated behavioral and modeling approach provides evidence suggesting how sensory modalities may be dynamically prioritized during adaptive behavior. This method offers a useful framework for

further exploring multisensory integration mechanisms at behavioral and potentially neural levels, enhancing our understanding of sensorimotor control.

Disclosures: O. Karagoz: None. **D. Uçar:** None. **A. Kolayli:** None. **M.M. Ankarali:** None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.10/T7

Topic: E.08. Multisensory Integration

Title: Roles of midbrain neurons in closed-loop control of refuge tracking in freely swimming weakly electric fish

Authors: *I. UYANIK[1], N. J. COWAN[2], E. S. FORTUNE[3];
[1]Hacettepe Univ., Ankara, Turkey; [2]Dept. of Mechanical Engin., Johns Hopkins Univ., Baltimore, MD; [3]New Jersey Inst. of Technol., Newark, NJ

Abstract: Natural behaviors are commonly controlled by feedback loops that use sensory slip, i.e., the relative shift between self-movement feedback and an external stimulus, to generate motor commands. We studied refuge tracking behavior in which weakly electric fish, a tracking behavior used to stabilize a moving refuge by determining the relative motion between body and refuge to generate compensatory locomotor commands. The locomotor plant, the ribbon fin, is mechanically low-pass, so theory predicts a neural controller with complementary high-pass dynamics (Cowan and Fortune, 2007). Specifically, we predicted that neurons would have neurophysiological properties that have a potential role in this 'error-correction' behavior. Such neurons would both respond to past tracking errors and drive future compensatory movements in the same direction. We tested this idea using neurophysiological recordings of midbrain neurons in freely swimming *Apteronotus leptorhynchus*.
We recorded 74 units in 13 fish while the subjects tracked a sinusoidally moving refuge (0-2 Hz). We measured sensory slip velocity, the error, and fish acceleration, the correction, via video tracking. Spike-triggered averages and direction-selectivity indices were calculated for past sensory input and future motor output.
Thirteen neurons appeared to have a role in error-correction, firing preferentially when past slip velocity and future fish acceleration were in the same direction. Their transfer functions were high-pass within the behaviorally relevant band (<2 Hz), matching control-theoretic predictions. Interestingly, selectivity differed depending on the tracking mode. Tracking modes are "exploit", also known as smooth pursuit, during which fish match the movement of the refuge, and "explore" mode, which includes epochs of active-sensing, during which fish produce movements for sensing that are not linearly related to the movement of the refuge. We observed increased selectivity during bouts of explore mode compared to exploit mode (paired *t* test, p=0.0033), driven by enhanced responses to faster slip velocities. 5 neurons selectively fired for headwards

movements, and the remaining 3 neurons for tailwards movements.
The other neurons either 1) had weak correlations with tracking behavior (n = 1), 2) were selective for error velocity but not fish acceleration (n = 1), or 3) responded to more complex combinations of sensory feedback and locomotor performance (n = 3). Of particular interest were neurons that responded to 'opposing' directions of sensory slip and fish acceleration. Such neurons may act to suppress incorrect movements or to generate active sensing movements.

Disclosures: I. Uyanik: None. **N.J. Cowan:** None. **E.S. Fortune:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.11/T8

Topic: E.08. Multisensory Integration

Support: TUBITAK Grant 120E198

Title: Adaptation of Active Sensing Responses to Sensory Manipulations in Weakly Electric Fish

Authors: *E. AYDIN[1], I. UYANIK[2];
[2]Electrical and Electronics Engin., [1]Hacettepe Univ., Ankara, Turkey

Abstract: Weakly electric fish such as *Eigenmannia virescens* continuously oscillate their bodies to actively shape electrosensory feedback. How these "active-sensing" movements reconfigure when sensory reliability degrades remains poorly understood. We challenged ten adult *E. virescens* with a refuge-tracking task in which the refuge followed sinusoidal trajectories (0.10, 0.15, 0.25 Hz) corrupted by four noise conditions (0, low, medium, high). Using a custom motorized shuttle and infrared video tracking, each fish (among N=10) experienced 120 open-loop trials (3 frequencies × 4 noise levels × 10 repetitions).As noise increased, fish displayed (i) larger amplitude active movements, (ii) reduced tracking gain, (iii) greater phase lag, (iv) lower correlation with the stimulus, and (v) higher root-mean-square error. These monotonic trends indicate a systematic shift in sensorimotor policy as the sensory scene becomes uncertain.To interpret the behavioral pattern, we implemented a family of control-theoretic models that embed predictive, stochastic, or mode-switching feedback strategies. Rather than seeking a single "best-fit" model, we use the ensemble to generate qualitative signatures—e.g., error-variance trade-offs, latency spectra—that can be matched to the experimental data. Preliminary analyses suggest that a hybrid controller combining state prediction with noise-dependent gain switching best reproduces the observed amplification of active movements.Together, the empirical and modeling results support the view that *E. virescens* dynamically reallocates control effort between predictive estimation and reflexive feedback to maintain tracking performance under uncertainty. These findings advance our understanding of robust active sensing and offer testable hypotheses for the neural circuits that mediate flexible sensorimotor control.

Disclosures: E. Aydin: None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.12/T9

Topic: E.08. Multisensory Integration

Support: TUBITAK Grant 120E198

Title: Dynamic Reweighting Visual and Electrosensory Feedback During Refuge Tracking in Weakly Electric Fishes

Authors: *C. S. OZDEMIR, N. DÜZ, I. UYANIK;
Hacettepe Univ., Ankara, Turkey

Abstract: Weakly electric fish adjust their motor output by combining visual and electrosensory information, offering a model for how sensory salience shapes control. We compared two wave-type gymnotiforms--*Apteronotus albifrons* and *Eigenmannia virescens*--to ask how environmental variables modulate this multisensory integration. Using a computer-controlled setup, each species was tested in 54 conditions created by factorial manipulation of four factors: illumination (dark, dim, light), refuge length (7, 14, 21 cm), refuge surface (windows present/absent), and water conductivity (low, medium, high). Custom 3D-printed PLA refuges followed a sum-of-sinusoids trajectory (0.10-2.05 Hz). Performance was quantified with four metrics: (i) root-mean-square error (RMSE) and (ii) variance in the time domain, and (iii) tracking error and (iv) active sensing error in the frequency domain.RMSE and tracking error captured smooth-pursuit accuracy across stimulus frequencies, whereas active sensing error highlighted exploratory movements when fish were not tightly coupled to the refuge. *A. albifrons* showed greater variability and stronger sensitivity to the manipulated environmental cues, while *E. virescens* maintained steadier tracking, implying different weighting of visual versus electrosensory feedback.The two species solve the same task with distinct cue-weighting strategies, illustrating how dynamic reweighting of sensory inputs underlies flexible motor control in changing environments.

Disclosures: C.S. Ozdemir: None. **N. düz:** None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.13/T10

Topic: E.08. Multisensory Integration

Support: TUSEB Grant 16548

Title: Optimal integration of visual and electrosensory cues during refuge tracking in Eigenmannia virescens

Authors: *E. RUTBIL[1], G. CELIK[1], O. KARAGOZ[2], I. UYANIK[1];
[1]Hacettepe Univ., ANKARA, Turkey; [2]Middle East Tech. Univ., ANKARA, Turkey

Abstract: Animals combine information from multiple senses to reduce uncertainty and guide action. Weakly electric fish provide a tractable model because electrosensory input can be varied independently of vision. We asked how *Eigenmannia virescens* weights visual and electrosensory cues when tracking a moving refuge. Adult fish (N = 5, 18-24 mo, 10-15 cm) tracked a translucent, motor-driven refuge. Electrosensory salience was manipulated by adjusting water conductivity (low vs. high) with a biocompatible salt mixture; visual salience was manipulated by projecting moving light stripes onto the refuge interior. Trials were run in darkness with or without visual cues. Position data were bootstrapped to create "virtual fish", increasing statistical power for frequency-response analysis. Cue reliabilities were entered into a Minimum Variance Unbiased Estimator (MVUE) to predict optimal multisensory behavior. Adding visual cues significantly improved tracking gain and responsiveness across stimulus frequencies, while non-tracking movements increased in the absence of visual input (two-way ANOVA, $p < 0.05$). MVUE predictions closely matched observed behavior, indicating that fish weight each modality in proportion to its experimentally defined reliability. *E. virescens* integrates visual and electrosensory information in a statistically optimal fashion, enhancing refuge-tracking accuracy when both cues are reliable. Independent control of cue salience and a bootstrapped analysis pipeline provide a powerful framework for probing the neural computations underlying multisensory integration.

Disclosures: E. Rutbil: None. **G. Celik:** None. **O. Karagoz:** None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.14/T11

Topic: E.08. Multisensory Integration

Support: TUBITAK Grant 120E054

Title: Multisensory Salience Shapes Zebrafish Target-Tracking Performance During Rheotaxis

Authors: *S. SOLMAZ, I. UYANIK;
Hacettepe Univ., Ankara, Turkey

Abstract: Effective navigation in flowing water demands that zebrafish (*Danio rerio*) combine mechanosensory and visual information. Yet how the salience of each cue influences this multisensory control remains unclear. We examined this question with a custom swim-tunnel assay in which a transparent D-shaped tube disrupted laminar flow to generate lateral-line input while a thin red rod inside the tube provided a coincident visual reference. Salience was parametrically manipulated across three dimensions: (1) ambient illumination (light vs. dark), (2) presence or absence of the outer transparent tube, and (3) diameter of the inner red rod. Three adult zebrafish were tested in every condition while the nested tube oscillated sinusoidally at several fixed frequencies. Fish trajectories were recorded, and tracking accuracy along with frequency-response characteristics were computed for each trial. Statistical comparisons showed a clear monotonic relationship: conditions that enhanced the clarity or availability of either modality yielded significantly tighter phase-locked tracking and higher overall gain. Conversely, depriving the fish of visual or mechanosensory clarity impaired performance, emphasizing the complementary roles of these channels. These findings demonstrate that zebrafish rheotaxis is not simply multisensory, but dynamically weighted by the perceived reliability of each input stream. Our results provide a tractable model for probing salience-dependent cue weighting in vertebrate sensorimotor integration.

Disclosures: S. Solmaz: None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.15/T12

Topic: E.08. Multisensory Integration

Support: JST CREST/JPMJCR22P5
JST CREST/CYC-MS2024005

Title: Effects of Visual Experience on the Formation of Spatial Orientation in Zebrafish

Authors: S. MIKI[1], M. TANIMOTO[2], S.-I. HIGASHIJIMA[2], ***Y. HIRATA**[1,3,4];
[1]Chubu Univ. Col. of Sci. and Engin., Aichi, Japan; [2]Exploratory Res. Ctr. on Life and Living Systems, Natl. Inst. of Natural Sci., Okazaki, Japan; [3]Chubu Univ. Ctr. for Mathematical Sci. and Artificial Intelligence, Aichi, Japan; [4]Chubu Univ. Acad. of Emerging Sci., Aichi, Japan

Abstract: Spatial orientation—the perception of self-motion, posture, and location within an environment—is established through the integration of multiple sensory modalities, particularly vestibular and visual inputs. Under clear visual conditions, these systems typically convey congruent information about self-motion. However, in natural environments where visual cues may be limited, ambiguous, or unreliable, this sensory congruency can be compromised. Such circumstances require flexible adaptation in spatial orientation strategies to ensure accurate perception and effective motor control. In this study, we investigated how visually structured

rearing environments influence the development of spatial orientation in zebrafish. Larvae were reared for 30-40 days post-hatching in cylindrical tanks surrounded by different visual patterns: vertical stripes, horizontal stripes, checkerboard patterns, or plain white backgrounds. These environments were designed to systematically manipulate the availability of optic flow cues generated by body tilt and rotation, thereby creating varying degrees of congruency between visual and vestibular signals. To assess the impact of these conditions on spatial orientation processing, we measured the vestibulo-ocular reflex (VOR), a compensatory eye movement that stabilizes the visual field and serves as an indicator of multisensory integration. Zebrafish reared in tanks with vertical stripes exhibited reduced vertical VOR gain during roll-axis rotation (about the rostral-caudal axis), while those raised with horizontal stripes showed reduced horizontal VOR gain during yaw-axis rotation (about the earth-vertical axis). These results suggest that when specific directions of head rotation are less visually discernible, the perceived magnitude of those rotations is diminished. In contrast, no significant VOR gain differences were observed in fish raised in checkerboard-patterned tanks, which provided salient omnidirectional motion cues, and in plain white tanks, despite the near absence of visual motion cues. Collectively, these results indicate that zebrafish adapt their strategies of spatial orientation formation according to the reliability of visual cues in their environment, underscoring the flexibility of multisensory integration during development.

Disclosures: S. Miki: None. **M. Tanimoto:** None. **S. Higashijima:** None. **Y. Hirata:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.16/T13

Topic: E.08. Multisensory Integration

Support: TÜBİTAK Grant 5240046

Title: A Radial Flow Tunnel to Study Multisensory Integration in Zebrafish During Rheotaxis

Authors: *N. DÜZ[1], M. KOCA[2], H. KAYIKÇI[2], I. UYANIK[3];
[1]Dept. of Electrical & Electronics Engin., Hacettepe University: Hacettepe Universitesi, Ankara, Turkey; [3]Electrical and Electronics Engin., [2]Hacettepe Univ., Ankara, Turkey

Abstract: Multisensory integration enables animals to build coherent percepts and select appropriate actions by combining information from different sensory channels. Yet, most neurobehavioral studies constrain animals physically, limiting the natural sensorimotor loops that shape perception in real life. Adult zebrafish offer an ideal model because they display robust rheotaxis—an innate behavior that relies on both mechanosensory (lateral-line) and visual cues. Harnessing this ethologically grounded behavior provides a powerful window into how sensory cues are reweighted when environmental salience changes.We developed a custom 360° radial rheotaxis arena that allows adult zebrafish to swim freely while the angle of flow changes during

the experiments. Radial flow eliminates directional bias, ensuring that mechanosensory stimulation is both continuous and precisely controllable. Flow speed is computer-controlled in real time, enabling systematic modulation of lateral-line input strength without disturbing the fish's natural posture or trajectory.A cylindrical LED screen encircles the arena, presenting high-contrast visual patterns that can be altered independently of flow. This design lets us manipulate visual salience—such as orientation cues or optic-flow speed—while holding mechanosensory conditions constant, or vice versa. High-speed videography tracks body kinematics and tail beats, providing a rich behavioral readout of sensory reweighting under fully unrestrained conditions.By coupling precise radial flow control with immersive visual stimulation, this assay isolates and recombines mechanosensory and visual signals in ways that mimic the complexity of natural streams while preserving experimental rigor. The platform sets the stage for dissecting neural circuits that mediate cue integration and for identifying principles of adaptive sensory weighting. Beyond zebrafish, the approach can be adapted to other small aquatic vertebrates, broadening its relevance to comparative studies of multisensory processing.

Disclosures: N. düz: None. **M. Koca:** None. **H. Kayikçi:** None. **I. Uyanik:** None.

Poster

PSTR395: Cross-Modal Processing: Spatial and Temporal Factors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR395.17/T14

Topic: E.08. Multisensory Integration

Support: TUBITAK Grant 120E054

Title: Independent Control of Visual and Mechanosensory Cues Reveals Multisensory Gains in Freely Swimming Zebrafish During Rheotaxis

Authors: S. ANILMAK, ***B. YUKSEKKAYA**, S. SOLMAZ, I. UYANIK;
Hacettepe Univ., Ankara, Turkey

Abstract: Animals rarely depend on a single sense; instead, they blend multiple sensory streams to reduce uncertainty and guide action. However, most vertebrate studies restrict the animal or stimulate only one modality at a time. We created a laminar flow-tunnel assay that independently delivers visual and mechanosensory stimuli to adult zebrafish *(Danio rerio)*, enabling quantitative study of multisensory integration during natural rheotaxis without restraint or sensory ablation. A dual-actuator system paired a blue LED strip (visual motion) with a transparent D-shaped tube that disrupted local flow (mechanosensation). Eight wild-type fish (mixed sex) were tested across five cue-conflict scenarios, including visual-only, mechanosensory-only, and synchronous bimodal stimulation. High-speed video was tracked with DeepLabCut, and body-position data were analyzed using frequency-response-function estimation with bootstrap resampling to assess variability. During synchronous bimodal stimulation, zebrafish exhibited significantly higher tracking gain and lower trial-to-trial

variability than in either visual-only or mechanosensory-only conditions ($p < 0.01$, $n = 8$). Freely swimming zebrafish actively fuse visual and mechanosensory information to stabilize locomotion, achieving more accurate and consistent target tracking than with either sense alone. The new assay offers a tractable platform for dissecting vertebrate multisensory mechanisms from behavior to neural circuits.

Disclosures: S. Anilmak: None. **B. Yuksekkaya:** None. **S. Solmaz:** None. **I. Uyanik:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.01/T15

Topic: F.02. Cerebellum

Support: UT Southwestern OBI Sprouts Grant
Simons Foundation Bridge to Independence Award
UT Southwestern CSTA Program CTSA-TPP-YR4-D-002

Title: Precision fMRI reveals domain-general and domain-specific neurocognitive effects of early cerebellar disruption

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Abstract: Emerging research suggests that the cerebellum may play a particularly important role in shaping early cognitive development. Cerebellar damage sustained earlier in development is associated with more severe cognitive impairments than damage sustained in adults. Importantly, there is variability in the degree and type of impairment following early cerebellar injury ranging from none to severe in domains such as language, theory of mind, and executive function. A better understanding of links between early cerebellar damage, neural reorganization, and cognitive outcomes could inform our basic understanding of the cerebellum's role in development with clinical implications. We recruited n=5 typically-developing (TD) adults and n=5 adults with early cerebellar damage (tumor resection at 3-5 yrs of age) for a precision functional MRI study to characterize effects of cerebellar damage during critical periods in cognitive development on the brain and behavior. Participants completed several cognitive fMRI tasks, including sentence listening, working memory, false belief, articulation, and motor behavior which robustly and reliably localize each individual's language, working memory, theory of mind, speech, and motor networks, respectively. Participants were also deeply phenotyped with a battery of neuropsychological assessments. We found individual differences in the brain and behavior across individuals ranging from impaired to minimal differences from

TDs. Importantly, nearly all cerebellar patients showed reduced scores relative to TDs on language (sight word reading, vocabulary) and executive function (fluency, rule learning, planning) measures. Despite diverse patterns of damage (to hemispheres vs midline), behavioral tasks requiring rapid task-switching were particularly affected. Individuals with greatest cognitive impairments also showed atypical activation in cerebro-cerebellar circuits typically engaged by these tasks. The observed results suggest that early cerebellar damage has distinct neurocognitive profiles depending on the subregion affected as reported in prior literature. However, we also find that early cerebellar damage might have more general effects in certain cognitive domains (i.e., language and executive function) that are emerging in the first years of life.

Disclosures: B. Wang: None. **M. Blanco:** None. **A. DeCurtis:** None. **A. Courreges:** None. **D.C. Bowers:** None. **A.M. D'Mello:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.02/T16

Topic: F.02. Cerebellum

Title: A cerebellar strategy to anticipate dopamine release

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Abstract: While the cerebellum is traditionally studied for its role in movement, increasing evidence links it to higher cognitive functions, such as reward prediction. Previous studies show many cerebellar granule cells (GrCs) display elevated ramping activity before an expected water reward, while climbing fiber (CF) inputs from the inferior olive spike only when water is given. We hypothesize that downstream Purkinje cells (PkCs) extract timing signals from GrCs and CFs to influence and reinforce appropriate reward seeking behavior. However, this has only been demonstrated with water reward that requires orofacial movements to consume. Thus, the question remains whether these signals represent rewarding outcomes or if they are present because orofacial muscles are engaged. To further link the cerebellum to reward, we sought to determine whether these cerebellar dynamics persist in anticipation of dopamine (DA) release. We trained mice (n=12) to self-initiate lever pushes for delayed electrical or optogenetic DA stimulation. Simultaneously, we imaged GrC calcium activity and CF spike rate using 2-photon microscopy. In a subset of mice (n=7), we compared activity between DA self-stimulation and water reward. As expected, orofacial movements observed before water reward were nearly absent prior to DA. Nevertheless, 19% of GrCs ramped in expectation of DA stimulation, comparable to 17% of GrCs that ramped in expectation of water. Further, 52% of CFs were

active at the time of DA stimulation, compared to 38% of CFs active at the time of water. We demonstrate that the cerebellum anticipates rewards using a common encoding strategy in anticipation of DA self-stimulation and water reward. These results solidify the cerebellum's involvement in reward processing, broadening our knowledge of cerebellar contribution to cognitive processes. This may inform novel targets in disorders impacting compulsive behaviors, such as in substance use disorders.

Disclosures: B.A. Filio: None. **M.J. Wagner:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: F.02. Cerebellum

Support: NS050808
DA044761
MH115604
RR027888

Title: Exploring the role of cerebellar contribution to dopamine signals during reward processing and flexible behavior

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Abstract: Dysfunction of the cerebellum (Cb) has been associated with a number of mental disorders where the dopamine (DA) system is dysregulated. However, the contribution of the Cb to DA signals in the mPFC remains to be established. Cortical DA encodes stimulus salience and reinforces rewarding actions, being required for flexible behavior. Cortical DA is provided by projections from the ventral tegmental area (VTA), a key region of the brain reward system. We have established the anatomical and functional properties of the Cb->VTA->mPFC circuit in the mouse brain, finding that neurons from all three nuclei of the Cb monosynaptically contact VTA neurons that project to mPFC, and that activation of Cb inputs to VTA increases the firing rate and dopamine levels at mPFC. To explore the behavioral role of this circuitry, we are using a reward-based decision-making task modified from the task implemented by the International Brain Laboratory. Head-restrained mice learned to use their front paws to steer a wheel that controlled the position of a visual cue displayed in the center of a digital screen placed in front of them. Mice needed to move the visual cue to one of the screen borders to receive a water reward.

At each trial, movements toward only one side were considered correct and rewarded (left or right), while movements to the opposite side triggered an error sound cue. The rewarded side was reversed when the mice reached a rate of 80% correct responses. Mice learn to switch their responses according to the rewarding rule, performing several (10 to 20) reversals per session. Inactivation with muscimol or block of DA receptors in the mPFC biased action selection, reduced correct responses to less than 50%, and made the mice incapable of successfully implementing rule reversal. Intriguingly, inactivation of the cerebellar vermis had comparable effects. Additionally, simultaneous fiber photometry recordings of GCaMP signals from Cb axons in the VTA and DA signals from mPFC show a high degree of correlation in mice performing this task. These preliminary results support the hypothesis that Cb contributes to DA signals in mPFC during reward processing and flexible behavior. We are conducting additional experiments to test this hypothesis thoroughly.

Disclosures: J.A. Vera: None. **M. Onate:** None. **C. Chen:** None. **L. Spaeth:** None. **J. Guarque-Chabrera:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Program #/Poster #: PSTR396.04/U2

Topic: F.02. Cerebellum

Support: NIH R01DA044761
NIH R01MH115604

Title: Midbrain-to-cerebellum dopaminergic and non-dopaminergic projections

Authors: *J. GUARQUE-CHABRERA[1], L. SPAETH[1], M. ONATE[1], J. A. VERA[1], F. NADIM[2,1], K. KHODAKHAH[1];
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Abstract: Traditionally implicated in coordinating high computational motor tasks, emerging evidence involves the cerebellum in several non-motor functions, including fear memory, cognitive flexibility, goal-directed behavior, rewarded behavior, and emotional processing. Moreover, cerebellar dysfunction relates to neuropsychiatric conditions such as autism, depression, schizophrenia, and substance use disorders. The cerebellar involvement in different functions and pathological conditions happens by its direct and indirect ascending activity modulation of diverse brain regions such as the medial prefrontal cortex, limbic areas, basal ganglia, midbrain, and the nucleus accumbens. On the other hand, descending pathways provide input to the cerebellum, and in this context, inputs via the pontine nuclei have received the bulk of the attention, sidelining extra-pontine direct cerebro-cerebellar pathways. Recently, the discovery of monosynaptic deep cerebellar nuclei (DCN)-to-midbrain projections has opened

new research avenues, particularly in regulating rewarded behaviors. However, whether a reverse midbrain-to-cerebellum connection exists remains a hotly debated topic. Here, we used neuro-anatomical tracing, optogenetics, and fiber photometry, to explore the presence and meaning of direct midbrain-to-DCN projections. Our neuroanatomical data suggests that all three DCN receive monosynaptic projections in a topographical distribution from different midbrain subregions. Moreover, midbrain optogenetic stimulation resulted in dopaminergic and non-dopaminergic transients in different cerebellar regions (DCN and cerebellar cortex) using fiber photometry recordings. Finally, we performed a Pavlovian licking task combined with fiber photometry recordings in the cerebellum to explore what information the midbrain conveys to the cerebellum in rewarded associative learning.

Disclosures: J. Guarque-Chabrera: None. **L. Spaeth:** None. **M. Onate:** None. **J.A. Vera:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

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Program #/Poster #: PSTR396.05/U3

Topic: F.02. Cerebellum

Support: NS050808
DA044761
MH115604
RR027888

Title: Anatomical organization of direct cerebellar projections to the midbrain and their disynaptic targets

Authors: *M. ONATE[1], J. A. VERA[1], L. KHATAMI[1], J. GUARQUE-CHABRERA[1], F. NADIM[2], K. KHODAKHAH[1];
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Abstract: The cerebellum is involved in a wide range of functions from motor control to cognitive behaviors and its dysfunction is associated with a variety of pathologies ranging from Dystonia and Parkinson's to mental disorders such as addictive behavior, schizophrenia, and autism. Our laboratory has previously characterized direct excitatory cerebellar (Cb) projections to the midbrain dopamine centers in the ventral tegmental area (VTA), Cb-VTA, and the substantia nigra pars compacta (SNc), Cb-SNc. Optogenetic activation of Cb-VTA results in dopamine release in downstream targets in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), as well as stimulation of Cb-SNc increases dopamine levels in the dorsal striatum (DS). Additionally, behavioral experiments in mice suggest that dopamine modulation by the Cb-VTA projection activates reward circuits and promotes social preference, while the

Cb-SNc projections may promote movement vigor. Yet, little is known about the anatomical details of these projections. Using different intersectional anatomical tracing experiments, we explore the organization of the specific pathways. We find that all three deep cerebellar nuclei (DCNs) contribute to cerebellar projections to the VTA and SNc, and both sets of projections arise from similar regions in each cerebellar nucleus. Analysis of the laterality of the projections suggests that cerebellar projections to VTA and SNc are bilateral, although mainly contralateral. By using Cre mouse lines we also mapped different cell types targeted by these projections. Furthermore, all three DCNs send disynaptic projections (via the VTA) throughout all the mPFC and NAc, and (via the SNc) to the medial and lateral DS. Retrograde tracing experiments in NAc and DS show that different regions of each nucleus are targeted by different VTA and SNc neurons, respectively. On the question of whether cerebellar midbrain projections arise as axon collaterals or if they are distinct projections, our functional and anatomical results suggest that Cb-VTA and Cb-SNc projections emerge primarily from non-overlapping and different populations of neurons, forming different sub-circuits.. Anterograde tracing from the midbrain neurons corroborates our previous results showing inputs from the midbrain back to the cerebellum. Characterization of collateral projections from the midbrain to downstream regions and the cerebellum complement our previous findings on new feedback loops between the cerebellum, the basal ganglia and the cortex. Our results suggest differential cerebellar modulation of the dopamine pathways in the brain involved in motor and non-motor behaviors.

Disclosures: M. Onate: None. **J.A. Vera:** None. **L. Khatami:** None. **J. Guarque-Chabrera:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.06/U4

Topic: F.02. Cerebellum

Support: NIH Grant R01NS105470

Title: Locus Coeruleus-dependent modulation of the cerebellum

Authors: *L. SPAETH[1], J. A. VERA[2], J. GUARQUE-CHABRERA[3], M. ONATE[5], F. NADIM[6], K. KHODAKHAH[4];
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Abstract: The cerebellum is involved in motor coordination, learning, and execution. In normal conditions, it enables the animal to perform smooth and accurate movements by predicting and steering the outcome of ongoing actions. Yet, numerous studies show that, in stressful situations,

proper movement execution can be impaired, thus pointing to a potential effect of stress on cerebellar function. However, mechanisms underlying stress-induced deficits in motor accuracy remain poorly understood, especially at the cerebellar level. In the brain, acute stress primarily activates the noradrenergic system, particularly the Locus Coeruleus (LC). The LC releases norepinephrine (NE), a neuromodulator known to regulate neuronal excitability and synaptic gain to promote vigilance and optimal performance. Using Slc6a2-Cre (NET-Cre) mice to selectively explore and manipulate the LC-NE projections, we found that the LC sends NE fibers to the entire cerebellum, suggesting that it has a large potential for exertion of its modulatory effects throughout the cerebellum. Furthermore, fiber photometry in freely moving animals showed that acute stressors (e.g. foot shocks) activate LC-NE neurons and their axons and trigger reliable elevation of NE concentrations in both the cerebellar cortex and deep nuclei. In the same animals, optogenetic activation of LC-NE neurons yielded comparable elevations of NE, suggesting that most of the stress-evoked noradrenergic increase in the cerebellum is driven by the LC. In addition, while NE concentrations and LC-NE activity are maximal in the couple of seconds following foot shocks delivery, they both remained higher than baseline for several minutes after, suggesting that NE may exert long-lasting modulatory effect overpassing the perception of the stimulus. Using a combination of different sensors in the same animals, we also found evidence suggesting release of Dopamine from the LC to the cerebellar cortex, but not to the cerebellar nuclei. Finally, specific expression of ChR2 in LC-NE neurons showed that optogenetic release of endogenous catecholamines from LC projections was sufficient to modulate the spontaneous firing in cerebellar Purkinje Cells. Taken together, our results provide a novel and promising framework to study how stress influences the neuronal computations and the physiology of the cerebellum.

Disclosures: L. Spaeth: None. **J.A. Vera:** None. **J. Guarque-Chabrera:** None. **M. Onate:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Topic: F.02. Cerebellum

Support: NIH Grant MH136675-01

Title: Interactions between the cerebellum and medial prefrontal cortex in rule-based category learning and rule-switching in rats

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Abstract: Previous findings indicate that patients suffering from posterior cerebellar lesions have impaired executive functions including lapses in working memory and task switching (Schmahmann and Sherman, 1998). In rodents, the posterior cerebellum has bidirectional communication with frontal cortical areas (prelimbic, infralimbic, anterior cingulate and orbital) via the lateral cerebellar nucleus (LCN). Category learning requires discrimination along relevant physical features (category-relevant dimension) and generalization along irrelevant features (category-irrelevant dimension). Previous research from our laboratory shows that NMDA lesions of the medial Prefrontal cortex (mPFC) disrupt rule-based (RB) category learning (Broschard et. al., 2019). Additionally, lesions and chemogenetic inhibition of the LCN disrupt learning of the same task, while chemogenetic excitation of the LCN facilitates learning. Based on these findings, we hypothesize that cerebellar communication with forebrain areas is essential for RB category learning. In the current study, we investigated the mechanisms of interactions between these regions using in-vivo electrophysiology in rats (n = 6). Using an operant chamber fitted with a touchscreen, rats were presented with circular stimuli that varied along two dimensions (spatial frequency and orientation) and were required to categorize the stimuli along a relevant dimension, while ignoring the other (e.g., high spatial frequency is category A, and low spatial frequency is category B). Rats were trained until they reached 75% accuracy on both categories, after which they received a multi-channel hyperdrive implant, with custom-made tetrode bundles targeting the LCN and mPFC. Subsequently, neuronal activity was recorded from both areas while rats performed the task. After 7 sessions, the relevant dimension (rule) changed, and the rats were trained to 75% accuracy or a maximum of 15 sessions. Neuronal activity was also recorded while the rats learned the second rule. We found that neurons in both regions show differential firing to the categories (i.e., category selectivity). LCN neurons show category selectivity during the first rule, which increases when the rule is changed. Similar results were observed for the mPFC as well, consistent with previous findings. These results support the role of the cerebellum's involvement in category learning and suggest communication with the mPFC.

Disclosures: S. Gupta: None. **K.L. Parker:** None. **J.H. Freeman:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.08/U6

Topic: F.02. Cerebellum

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HHMI
Raynor Cerebellum Project

Title: Cerebellar adjustment of neocortical dynamics during non-motor learning

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Abstract: The cerebellum facilitates smooth, coordinated, and adaptable movements by forming internal models that predict the sensory consequences of actions. Through interactions with motor regions of the brain, these predictions enable us to correct errors in our movements and adapt to new environments. We hypothesize that the cerebellum serves a similar function for cognition by forming internal models that error-correct and adapt neocortical dynamics related to non-motor processes. To test this hypothesis, we developed a timing task for macaque monkeys in which they use the timing of a predictable stimulus—termed the "flash"—to correct errors in their internal time-keeping and adapt to changes in the temporal environment. Behavioral data from two monkeys suggest that they rely on an internal model to predict the timing of the flash and use sensory prediction errors to rapidly correct their internal clock. Moreover, the monkeys integrate prediction errors across trials to adapt to blocked changes in the flash time.
To understand whether and how the cerebellum corrects and adapts timing-related dynamics in neocortex, we combined large-scale recordings, perturbations, and circuit-mapping throughout the cerebello-thalamocortical pathway during the task. Simultaneous recordings with neuropixels in the dentate nucleus (DN), its target in the ventral lateral thalamus (VL; identified via orthodromic stimulation), and the lateral intraparietal area (LIP) are ongoing. Preliminary results suggest that LIP displays ramp-to-threshold dynamics that predict timing behavior and undergo an error-correction induced by the flash. Specifically, the speed of ramping after the flash depends on whether the flash came earlier or later than the monkey expected. In contrast, VL displays tonic activity, the level of which tracks the monkey's estimate of the current interval. In DN, the majority of neurons burst or suppress their activity in response to the flash. The magnitude of this modulation depends on temporal prediction error and predicts the degree of error-correction of ramping dynamics in LIP. Finally, electrical microstimulation of DN decreased the monkey's timing variability when it occurred at the expected time of the flash, mimicking error-correction. The results offer a first glimpse into how the cerebellum uses internal models to control internal, non-motor processes in the neocortex.

Disclosures: G.M. Stine: None. **B. Zheng:** None. **M. Jazayeri:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

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Topic: F.02. Cerebellum

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Title: Cerebellum-driven plasticity in the VTA underlies depression-like behaviors induced by chronic stress

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Abstract: In line with the growing understanding of the cerebellar involvement in cognition and emotion, our previous research demonstrated that cerebellar output neurons contribute to chronic stress-induced depression-like behaviors. Specifically, neurons in the dentate nucleus (DN) exhibited transient activation upon restraint stress (RS), and repeated activation of DN neurons projecting to the ventral tegmental area (VTAp-DN neurons) during chronic RS promoted the development of depression-like behaviors. To investigate the downstream mechanisms by which VTAp-DN neurons influence these behaviors, we examined synaptic molecule changes in the dorsolateral VTA — the primary target of DN projection — following acute (3 days, ARS) and chronic (14 days, CRS) RS. We found a significant reduction in vesicular glutamate transporter 2 (VGLUT2) expression in DN neuron axon terminals after CRS, but not after ARS, which did not elicit depression-like behaviors. Consistently, the frequency of miniature excitatory postsynaptic currents (mEPSCs) recorded from dopaminergic neurons in the dorsolateral VTA was also diminished. To assess whether these reductions depended on VTAp-DN neuron activity, we employed chemogenetic inhibition of these neurons during CRS. This manipulation reversed both reductions in VGLUT2 and mEPSC frequency. Importantly, the reduction in VGLUT2 persisted for at least 10 days after the end of CRS, a period during which depression-like behaviors were sustained, suggesting a potential causal link between VGLUT2 reduction and behavioral outcomes. In fact, targeted overexpression of VGLUT2 in VTAp-DN neurons significantly reduced depression-like behaviors, even after CRS, emphasizing the relevance of presynaptic regulation in VTAp-DN neurons. Taken together, our findings indicate that repeated activation of cerebellar VTA-projecting DN neurons during CRS induces plastic changes in downstream VTA circuits that contribute to the manifestation of depression-like behaviors. These results raise the possibility that presynaptic adaptations in cerebellar projections to the VTA may serve as a potential target for alleviating stress-related disorders.

Disclosures: S. Kang: None. **T. Kim:** None. **D. Kim:** None. **S. Baek:** None. **Y. Yamamoto:** None. **K. Tanaka-Yamamoto:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Title: Reversal learning in adolescent mice is modulated by lateral cerebellar nuclei parvalbumin enwrapped perineuronal nets

Authors: *T. LYLE[1], J. L. VERPEUT[2];
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Abstract: Autism spectrum disorder (ASD) clinical evidence has found atypical connectivity between the cerebellum and distal cortical regions associated with cognition. The most common atypical brain structure in ASD, the cerebellum, has multisynaptic connections through the cerebellar nuclei (CN) and thalamus to cognitive-associated brain regions (Pisano et al., 2021; Verpeut et al., 2023), yet formation of these pathways are not fully understood. Moreover, we previously found reducing lateral CN (LCN) perineuronal nets (PNNs), specialized extracellular matrix structures whose appearance is associated with the end of the critical period of plasticity and enwrap fast-spiking parvalbumin (PV) interneurons, improved reversal learning in a visual discrimination task (Lyle and Verpeut, 2025). Moreover, LCN manipulated mice were found to have decreased cFos, an immediate early gene, in the ventral tegmental area, nucleus accumbens, and anterior cingulate cortex (ACC). Additionally, mice that received the excitatory DREADD perturbation showed increased ACC dendritic complexity. However, the extent to which CN PV activity changes with PNN quantity and how these changes in plasticity influences adolescent cognitive behavior during learning is currently unknown. We hypothesized that typical maturation of CN PNNs is required to shape PV cell activity and the cerebello-neocortical circuit, which is essential for flexible learning. In the following experiments, adolescent male C57BL/6J mice CN activity was reduced using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) (AAV2-hSyn-hM4D(Gi)-mCherry) or chondroitinase ABC (ChABC). All mice received the DREADD ligand, clozapine-N-oxide (CNO; 10 mg/kg), in drinking water from postnatal day 21-35. Animals also received an injection of GCaMP (AAV2-S5E2-GCaMP6f) to drive calcium expression in CN PV cells. PV calcium activity was recorded using the Plexon Multi-Wavelength Photometry System while animals underwent acquisition and reversal learning of the visual discrimination task. Post-DREADD manipulation, local LCN GCaMP (AAV1-hSyn-jGCaMP8f-WPRE) signaling was disrupted compared to control animals, suggesting an adolescent CN manipulation impacts both local and long distance signaling. Our results suggest that delaying or reopening LCN critical periods via PNNs, can prolong formation and maturation of CN-ACC circuit development which can in turn improve adolescent flexible learning. These findings can guide clinical applications targeting development in individuals with neurodevelopmental disorders, such as ASD.

Disclosures: T. Lyle: None. **J.L. Verpeut:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

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Topic: F.02. Cerebellum

Support: NINDS NCFA
NIH Intramural Research

Title: Task-separation and generalization in cortico-cerebellar circuits

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Abstract: Learning and switching between multiple tasks is fundamental to adapting in changing environments. Unlike the brain, artificial neural networks are notoriously susceptible to catastrophic forgetting: learning new tasks degrades performance on previously learned tasks. In the brain, the neocortex and the cerebellum are thought to implement computations that underlie higher learning. These two areas specialize in distinct roles, but also synergize through dense interconnected loops. Recent computational studies posit that cortico-cerebellar loops could help learn to acquire and switch between several tasks. Yet to date, experimental evidence is lacking, due to the technical difficulty of repeatedly recording from fixed sets of cortical and cerebellar neurons simultaneously, across the weeks required to learn multiple new skills. Here, we developed a two-photon microscopy strategy to simultaneously image activity in fixed populations of individual premotor cortex layer 5 neurons (L5N) and cerebellar granule cells (GrC), while mice learn two behavioral tasks in parallel: (1) reach for a target using a manipulandum, and (2) run towards a target in virtual reality, both for water reward. While each task requires distinct actions, both share a 1 s delay period between action completion and reward, in which mice learn anticipatory licking. Previously, we showed that GrCs develop activity that ramps at different rates during this delay, which was essential for learning the timing of reward. Here, we found that optogenetic inhibition of GrCs in 3 expert mice during the delay disrupted anticipatory licking in both tasks. Imaging fixed populations of both L5Ns and GrCs from novice to expert in 5 mice revealed that both L5Ns and GrCs developed strong and stable neural representations of both tasks. When comparing cells, however, we found that individual L5Ns often activated similarly across tasks, while individual GrCs often varied their activity widely between tasks. These results suggest that the cerebellum could facilitate task separation to avoid interference between the two tasks, as they share behavioral outcomes (i.e. anticipatory and consummatory licking). We hypothesize that task-separation and generalization mechanisms differ between the neocortex and the cerebellum, likely reflecting their distinct neuronal architectures, and plasticity and learning rules. Additional theory and modelling will inform on how these structures jointly achieve multi-task learning. Simultaneous, dual-site neuronal recordings during multiple task learning could uncover computations supporting complex behavior and inspire improved artificial neural networks.

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Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Program #/Poster #: PSTR396.13/U10

Topic: F.02. Cerebellum

Support: NSTC 112-2628-B-002-011
NSTC 113-2628-B-002-002

Title: The cerebellum encodes the kinematic frequencies of motion directly via cerebellar-spinal pathways

Authors: *K. FANG[1], M.-K. PAN[2];
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Abstract: Our brains control our motion with supreme precision. However, even with the support of AI, direct recording from primary motor cortex (M1) is not sufficient to decode motor-control details. Here we reported that the kinematic details of motion are directly transmitted from the cerebellum to the spinal cord. Using in-vivo single unit recordings and optogenetics in freely moving mice, we optogenetically controlled populational firings of neurons in deep cerebellar nucleus (DCN) and generated motions with designed frequency dynamics. However, similar approach at M1 was not able to generate related motions. We investigated rhythmic motions, pattern motions of gait, and skilled reaching movements, all revealed that M1 simulation or silencing may alter the motion initiation but could not change kinematic details. In contrast, cerebellar modulation significantly disturbed abovementioned movements. Viral-based anatomical tracing revealed that DCN neurons sent axonal terminal into spinal cord. Optogenetic simulation at these axonal terminals sufficiently generate complex kinematic details in mice. Electroencephalography (EEG) in healthy subjects with volitional rhythmic tapping confirmed that frequency-dependent kinematic details were observed in the cerebellar regions but not in M1. Our discovery suggests the cerebellum transmits motor kinematic codes directly to spinal cord independent of primary motor cortex.

Disclosures: K. Fang: None. **M. Pan:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.14/U11

Topic: F.02. Cerebellum

Title: Tremor tolerance in essential tremor is driven by frequency-independent signal leakage through the cerebello-thalamo-cortical circuit

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Abstract: Essential tremor (ET) is a common neurological disorder characterized by involuntary rhythmic movements originating from cerebellar dysfunction, affecting nearly 20% of the elderly. However, given the limited efficacy of currently available pharmacological treatments, clinical interventions such as thalamotomy and deep brain stimulation (DBS) still face major challenges of therapeutic tolerance and tremor recurrence. In our previous works, cerebellar oscillations have been confirmed as the source of tremor generation. Here, we investigated the propagation of tremor through the cerebello-thalamo-cortical (CTC) circuit using in vivo electrophysiology, optogenetics, and motion tracking in Grid2dupE3 and wild-type mice. We report that tremor generation depends on CTC in a frequency-independent manner. While microinfusion of lidocaine into the thalamus could disturb CTC rely and stop tremor in Grid2dupE3 mouse, tremor reappeared faster in the washout state while oscillation in the primary motor cortex remained suppressed. During optogenetic suppression of cerebello-thalamic axons, an 8% frequency-independent leak of axonal signals to thalamus sufficiently recreated a full-blown tremor. Optogenetic thalamic stimulation failed to induce tremor in wild-type mice or alter tremor frequency in Grid2dupE3 tremor mice, while cerebellar oscillations reliably elicited tremor via the olivocerebellar-spinal pathway. To evaluate whether this frequency-independent neuronal signaling via CTC pathway contributes to tremor tolerance in DBS, we investigated thalamic DBS effects in tremor mice. Thalamic DBS effectively suppressed tremor but led to symptom relapse associated with a frequency-independent signal leakage. Our findings reveal that cerebellar information flow via CTC is necessary for generating tremor. However, it is in a frequency-independent manner. This mechanism allows tremor relapse during DBS with a minor, frequency independent leak during disease progression, potentially explained the mechanism of therapeutic tolerance in DBS therapy.

Disclosures: T. Liang: None. **M. Pan:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

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Topic: F.02. Cerebellum

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Title: Cerebellar tDCS effects on the laterality of visual rhythm perception: A preliminary study

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Abstract: Rhythm perception requires temporal prediction of the next stimulus from the repetitive stimuli. The cerebellar dentate nucleus (DN) plays an important role in its processing. A previous study showed that the unilateral DN mainly processes the ipsilateral visual rhythm perception compared with the contralateral one, suggesting the laterality of the rhythm perception processing. In the present study, we investigated the effects of cerebellar transcranial direct current stimulation (tDCS) on the laterality of rhythm perception. Seven right-handed healthy adults (age 26.4 ± 3.2 years; 2 females) participated in this study. Anodal or cathodal tDCS was applied on the left cerebellum (3×3 cm) in separate days (reference electrode on the right trapezius muscle, 2 mA, 20 minutes). The tDCS was given concurrently with the missing oddball task for investigating the laterality of rhythm perception and motor response: a pre-stimulus indicating movement direction was presented on either the left or right side of the monitor, and subsequently, repetitive visual stimuli (2.5 Hz) were presented on either the left or right side. The participants had to press the left or right or key indicated by the motor stimulus as soon as they detected an omission of the visual stimuli. Reaction time (RT) was recorded before, during and after the intervention according to the directions of motor and visual stimuli (32 trials per condition in each period). RT improvement rates were calculated to compare between each side of the visual stimuli (Visual Left: VL vs. Visual Right: VR) in each motor direction (paired t-test). As a result, in the left motor condition, cathodal tDCS improved the RT in the VL compared with the VR after the stimulation ($p = 0.016$). In the right motor condition, there was no improvement on both sides ($p > 0.05$). Anodal tDCS did not show any improvement ($p > 0.05$). This study suggests that cathodal tDCS over the cerebellum likely improved performance in the ipsilateral rhythm perception resulting in the improved movement in the ipsilateral side possibly by reducing inhibition to the ipsilateral DN from the Purkinje cells in the stimulated cerebellar cortex.

Disclosures: S. Kawakami: None. **T. Mima:** None. **S. Koganemaru:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Program #/Poster #: PSTR396.16/U13

Topic: F.02. Cerebellum

Support: JST CREST/JPMJCR22P5
JSPS KAKENHI/24H02338

Title: Differential Contributions of the Cerebellum and Brainstem Area II to Predictive Visually Evoked Eye Movements in Goldfish

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Abstract: The optokinetic response (OKR) is a reflexive eye movement that stabilizes retinal images during visual motion. In goldfish, repeated exposure to visual stimuli that reverse direction in a predictable manner induces a learned form of OKR, known as predictive OKR (pOKR) (Marsh & Baker, 1997). Once acquired, the eyes begin to decelerate in anticipation of the upcoming reversal—a phenomenon termed predictive deceleration. Remarkably, even in complete darkness, trained fish exhibit rhythmic eye velocity patterns that reflect the learned timing of the visual stimulus, referred to as predictive oscillations. The cerebellum is essential for both the acquisition and expression of pOKR as cerebellectomized goldfish fail to acquire pOKR, and cerebellectomy after learning abolishes the predictive response (Miki et al., 2018). During predictive oscillations, Purkinje cells in the vestibulo-cerebellum fire in sync with eye velocity. However, the origin of the oscillatory signals modulating Purkinje cell responses in the absence of visual input remains unclear. One candidate structure is Area II in goldfish, a brainstem region that contains neurons sensitive to both eye and head velocity and is functionally analogous to the nucleus prepositus hypoglossi (NPH) in mammals (Beck et al., 2006). It has demonstrated that Area II sends major mossy fiber inputs to the vestibulo-cerebellum (Straka et al., 2006). This anatomical and functional connectivity raises the possibility that Area II participates in generating the internal timing signals required for pOKR. To test this hypothesis, we recorded extracellular activity from Area II neurons in goldfish during the test phase of a pOKR paradigm, in which predictive oscillations are observed in complete darkness. Remarkably, Area II neurons exhibited rhythmic firing patterns that closely aligned with the ongoing predictive eye velocity, despite the absence of visual input. This suggests the presence of an internally generated timing mechanism within Area II, operating independently of immediate sensory cues. Taken together with its extensive mossy fiber projections to the cerebellum, these findings suggest that Area II provides critical temporal input that enables the cerebellum to generate predictive motor output. Thus, predictive eye movements in goldfish likely emerge from coordinated activity of a distributed network involving the cerebellum and brainstem regions such as Area II.

Disclosures: T. Yamanaka: None. **Y. Hirata:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Program #/Poster #: PSTR396.17/U14

Topic: F.02. Cerebellum

Support: NIH Grant R01-HD095959
NIH Grant T90AR085527

Title: Cerebellar Structural Differences and Clinical Correlates in Chronic Low Back Pain

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Abstract: Pain is a multidimensional experience that is processed and represented by a matrix of cortical and subcortical structures. Accumulating neuroimaging evidence describes associations between the cerebellum and both acute and chronic pain. Lobules IV-VI and Crus I have consistently been implicated in the literature as pertinent to pain processing, and these structures have anatomical and functional connections with cortical structures related to cognitive, emotional, and sensorimotor function. We tested whether this finding translates to a cohort of individuals with chronic low back pain (cLBP). We compared cerebellar gray matter volume (GMV) in lobules IV-VI and Crus I between 53 individuals with cLBP and 48 matched healthy controls (HC). Additionally, we evaluated the relationship between cerebellar GMV and self-report measures of pain, function, kenisiophobia, and pain catastrophizing in the cLBP group. High-resolution T1-weighted structural images were acquired on a 3T MRI scanner. A cerebellum and brainstem optimized volumetric analysis was conducted using the spatially unbiased infratentorial template (SUIT) toolbox implemented in Statistical Parametric Mapping software, version 12. An anatomical atlas of the cerebellum was employed; GMV measures from each region were normalized to total intracranial volume. To compare GMV between cLBP and HC groups, we used ANCOVA models, controlling for age and sex, and calculated Hedge's g effect sizes. Within the cLBP group, we evaluated the relationship between GMV and self-report measures using partial correlations controlling for age and sex. We found no differences between groups for lobules IV-VI. However, we identified significantly lower GMV in the cLBP group for Crus I. Previous work has suggested that Crus I is involved in sensorimotor integration, cognitive pain modulation, and affective-motivational processing via connectivity with sensorimotor, default-mode, salience, and reward networks. Within the back pain group, we identified a significant negative correlation between pain interference and GMV in the vermis of lobule VI. Hence, lower GMV was associated with higher pain interference for individuals with cLBP. Lobule VI has both afferent and efferent connections to the primary motor cortex, in addition to connections to prefrontal and limbic areas previously implicated in cLBP. Together, our results highlight Crus I and lobule VI as key cerebellar regions linked to chronic pain and extend these findings to individuals with cLBP. Data support the potential of therapeutic approaches that target the cerebellum for chronic pain.

Disclosures: J.R. Gilliam: None. **Y. Shin:** None. **S.P. Silfies:** None. **S. Coombes:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.18/U15

Topic: F.02. Cerebellum

Support: NSTC 113-2320-B-002-052-
NSTC 113-2320-B-002-001-

Title: Alpha 6 containing-gaba$_A$ receptor-selective positive allosteric modulator as a potential therapy for autism spectrum disorder: a preclinical study in fmr1 ko mice

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition with both genetic and environmental risk factors. Our previous study (Pan et al., 2024) revealed an unprecedented role of cerebellar α6 subunit-containing GABA$_A$ receptors (α6GABA$_A$Rs) in ASD pathogenesis using DK-I-56-1 (DK), an α6GABA$_A$R-selective positive allosteric modulator (PAM) of the pyrazoloquinolinone (PQ) class, in a valproic acid-induced rat model representing environmental risk. Here, we utilized the *Fmr1* knockout (KO) mouse model, an established ASD genetic model, to further evaluate DK's therapeutic potential across both etiological backgrounds. Given that the *Fmr1* gene is an X-linked risk gene of ASD, we tested 5-6 week-old male hemizygotes (*Fmr1*-/y), female hemizygotes (*Fmr1*-/+), and female homozygotes (*Fmr1* -/-) as disease groups, and their sex-matched wildtype littermates as controls. ASD-like behaviors were assessed using the three-chamber social interaction test for sociability and social preference, and the open field test for repetitive and anxiety-like behaviors. Results showed a gene-dose effect of *Fmr1* in maintaining normal social functioning. Both male and female hemizygotes exhibited reduced sociability and social preference, with males showing more pronounced deficits in sociability than females. Moreover, in females, homozygotes showed greater impairments in both social preference and sociability than hemizygotes. DK (10 mg/kg, *i.p.*) administered once daily for five consecutive days significantly rescued ASD-like behavior across genotypes and sexes. Moreover, DK markedly reduced gene dose-dependent repetitive and impulsive behaviors in female *Fmr1* KO mice, assessed by marble burying and elevated plus maze tests. Western blot analysis of whole cerebellar tissues of *Fmr1* KO mice revealed a reduction in the α6 subunit expression, reflecting diminished GABAergic transmission at Golgi cell-granule cell synapses. This may enhance GABAergic inhibition of Purkinje cells (PCs) by molecular layer interneurons, as suggested by increased glutamate decarboxylase 65 and decreased GABA transporter 1 levels also observed in Western blot analysis. These findings support a disrupted excitation-inhibition balance in the cerebellar cortex of *Fmr1* KO mice, characterized by weakened GC input and upregulation of inhibitory signaling onto PCs. By potentiating α6GABA$_A$R function, DK can strengthen GABAergic transmission in GCs, restore PC activity, and ameliorate ASD-like symptoms. With favorable pharmacokinetics, including good oral bioavailability, a 13-hour half-life, and no sedative or abuse potential, DK is a promising candidate for treating ASD.

Disclosures: Y. Lee: None. **D. Sharmin:** None. **J.M. Cook:** None. **S. Chiu:** None. **L. Chiou:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.19/U16

Topic: F.02. Cerebellum

Support: NSTC 113-2320-B-002-052-
NSTC 113-2320-B-002-001-

Title: α6GABA$_A$ receptor-selective positive allosteric modulator as a potential autism therapy via purkinje cell restoration in prenatal valproic acid-exposed juvenile rats

Authors: *Y.-T. PAN[1,2,3], C.-C. WU[4], M. CHU[4], C.-J. YEH[3], D. SHARMIN[5], J. M. COOK[5], H.-C. LIN[4], L.-C. CHIOU[3,6];
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Abstract: Autism spectrum disorder (ASD), a neurodevelopmental condition characterized by social deficits and repetitive behaviors, affects approximately 1% of children worldwide and remains an unmet medical need. Cerebellar dysfunction, particularly the loss or hypofunction of Purkinje cells (PCs), has been implicated in its pathophysiology. We previously identified pyrazoloquinolinone DK-I-56-1 (DK) as a druggable positive allosteric modulator (PAM) selective for α6 subunit-containing GABA$_A$ receptors (α6GABA$_A$Rs), which are primarily expressed in cerebellar granule cells (GCs) that directly excite and indirectly inhibit PCs. We hypothesize that DK reduces indirect inhibition to PCs, thereby restoring their function and number and ameliorating ASD-like symptoms. To test this hypothesis, we employed a prenatal valproic acid (VPA) exposure rat model of ASD. Pregnant rats were administered VPA (500 mg/kg, *i.p.*) or saline on E12.5. Their offspring's social behaviors were assessed by the three-chamber social interaction test on P28, and repetitive behavior by the open-field test on P29. Male offspring from the VPA group exhibited significant social deficits and increased repetitive behaviors, whereas females did not. Therefore, therapeutic efficacy of DK was evaluated in males across four groups: Saline/Veh, Saline/DK, VPA/Veh, and VPA/DK. A single injection of DK (10 mg/kg, *i.p.*) during the juvenile period significantly improved sociability and reduced rearing behavior in VPA/DK rats compared to VPA/Veh rats. Furthermore, while a three-day DK regimen was insufficient, five consecutive days of DK treatment fully normalized autism composite scores, calculated as the average z-score of social and repetitive behaviors, to levels

comparable to Saline/Veh rats. To investigate DK's therapeutic mechanism, cerebellar vermis tissues were stained for calbindin, a PC specific marker. PC numbers were significantly reduced in VPA/Veh rats, particularly in lobules VII and IX, and this loss was prevented by DK treatment. Additionally, PC activity, assessed by c-Fos expression, was markedly decreased in lobule VII of VPA/Veh rats and restored by DK. Pre-administered of furosemide (10 nmol), an α6GABA$_A$R antagonist, into lobule VII prior to DK treatment blocked its preventive effects on ASD-like behaviors, confirming that DK acts via α6GABA$_A$Rs in this region. These findings suggest that DK provides both therapeutic and preventive benefits in ASD, likely via restoring PC function and preventing PC loss in cerebellar vermis lobule VII. Our study supports α6GABA$_A$R PAMs as promising candidates for targeting core symptoms of ASD.

Disclosures: Y. Pan: None. **C. Wu:** None. **M. Chu:** None. **C. Yeh:** None. **D. Sharmin:** None. **J.M. Cook:** None. **H. Lin:** None. **L. Chiou:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.20/V1

Topic: F.02. Cerebellum

Support: NSTC 113-2320-B-002-052-
NSTC 113-2320-B-002-001-

Title: An Unprecedented Role of Cerebellar α6GABA$_A$ Receptors in Morphine-Conditioned Place Preference: A Preclinical Study Revealing DK-I-56-1's potential for Treating Opioid Use Disorder

Authors: *L.-C. CHIOU[1], Y. LEE[2], J. HSU[2], D. SHARMIN[3], J. M. COOK[3];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Pharmacol., Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [3]Chem. and Biochem., Univ. of Wisconsin Milwaukee, Milwaukee, WI

Abstract: Opioids are indispensable for pain management but have contributed to a growing global public health crisis, with opioid use disorder (OUD) accounting for nearly 75% of overdose-related deaths. Although current therapies are available, their limited long-term efficacy underscores the need for new therapeutic strategies. Emerging evidence suggests a critical role for the cerebellum in cognitive dysfunction and maladaptive social behaviors, particularly through projections from the deep cerebellar nuclei (DCN) to the ventral tegmental area (VTA), a major source of dopaminergic input to the nucleus accumbens (NAc) within the reward circuitry. Within this framework, cerebellar granule cells (GCs) influence cerebellar output, and their α6 subunit-containing GABA$_A$ receptors (α6GABA$_A$Rs) have been implicated in various neuropsychiatric disorders. In this study, we examined whether α6GABA$_A$R-selective positive allosteric modulators (PAMs), specifically pyrazoloquinolinone (PQ) Compound 6 and its deuterated derivative DK-I-56-1, could be a potential therapy of OUD in male ICR mice with

morphine-conditioned place preference (CPP). Pre-treatment with Compound 6 prior to conditioning significantly prevented CPP acquisition. This effect was abolished by intra-cerebellar blockade of α6GABA$_A$Rs by furosemide and was nullified in Gabra6-knockout mice, confirming the involvement of cerebellar α6GABA$_A$Rs. DK-I-56-1 pretreatment also prevented CPP acquisition, and a single-dose intervention effectively inhibited established CPP. Western and immunofluorescence studies revealed that repeated morphine dosing resulted in cerebellar α6GABA$_A$R down-regulation, reduced number of activated Purkinje cells, and increased neuronal activity in the DCN. DK-I-56-1 pre-treatment, but not a single dose, restored α6GABA$_A$R expression. However, a single dose of DK-I-56-1 restored Purkinje cell activity and inhibited DCN overactivation. which may contribute to its inhibition of morphine-seeking behavior. Mechanistically, DK-I-56-1 inhibition on DCN neurons can lead to a suppression of DCN-VTA output and subsequent dopamine release in the NAc shell, suggesting the involvement of cerebellar α6GABA$_A$Rs in regulating reward-seeking behaviors. These findings uncover a novel role of cerebellar α6GABA$_A$Rs in opioid reward processing and identify α6GABA$_A$R-selective PAMs as promising candidates for OUD treatment. By bridging cerebellar function with addiction neurobiology, this study supports a circuit-based therapeutic approach to combat opioid addiction.

Disclosures: L. Chiou: None. **Y. Lee:** None. **J. Hsu:** None. **D. Sharmin:** None. **J.M. Cook:** None.

Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.21/V2

Topic: F.02. Cerebellum

Support: The Nemours Foundation
NIH Grant P30GM145765

Title: Motor deficiencies and altered social behavior in mice with deletion of Na,K-ATPase beta$_1$-subunit in cerebellar granule cells

Authors: K. SPERLE[1], M. RASHEED[1], M. KHALIFE[2], A. CORT DONMEZ[1], A. E. HERNAN[3], ***S. A. LANGHANS**[1];
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Abstract: The Na,K-ATPase is an ubiquitous ion pump consisting of two core subunits, the catalytic α-subunit and an accessory β-subunit. Aside from its pump function crucial for maintaining ion homeostasis and membrane potential, the Na,K-ATPase exhibits pump-independent roles as a signaling scaffold and its β_1 and β_2-subunits function as cell adhesion

molecules. The α-subunit has been implicated in neurological disorders associated with the cerebellum. However, the significance of its β-subunit in neuronal function is unknown. To elucidate whether the β_1-isoform plays a specific role in cerebellar development and neurodevelopmental disorders, we selectively deleted the β_1-subunit (β_1) in cerebellar granule cells (CGCs) in mice. CGCs integrate signals to modulate motor functions and participate in non-motor behaviors such as emotion, abstract thinking, and spatial navigation.The absence of β_1 mRNA and protein in the cerebellum of knockout (KO) mice was confirmed by cerebellar genomic DNA analysis and immunoblotting. Morphological changes in the cerebellum of KO mice were assessed by histology, immunohistochemistry, transmission electron microscopy, and MRI. Heterozygous KO mice performed Barnes maze tests for learning and memory and social interaction and catwalk experiments for motor functions. Deletion of the β_1 in CGCs resulted in complex neurological deficits. Homozygous KO mice displayed symptoms consistent with cerebellar ataxia that worsened with age. They also were significantly smaller and had a shorter lifespan compared to heterozygous KO mice or control littermates. At first sight, heterozygous KO mice appeared normal with a normal lifespan and no motor abnormalities. However, they exhibited deficiencies in the Barnes maze tasks reminiscent of an autism-like phenotype. H&E staining did not reveal any discernible differences in CGCs or the overall cerebellar architecture between the homozygous KO, heterozygous KO, and control littermate mice. Ex vivo, high-resolution MRI of the cerebellum confirmed the lack of significant structural differences in KO mice, although a reduction in cerebellar volume in homozygous KOs was observed.Overall, targeted knockout of β_1 in CGCs results in a dosage-dependent phenotype. Heterozygous KO mice displayed deficits in social behavior and homozygous KO mice had progressive cerebellar ataxia. Interestingly, the phenotype observed in mice lacking the β_1 was markedly distinct from mice with deletion of the β_2-isoform. Thus, the β_1-isoform plays a crucial and unique role in CGCs and cerebellar development.

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Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.22/V3

Topic: F.03. Basal Ganglia

Support: JSPS KAKENHI Grant JP22K06475

Title: Red nucleus and its neuronal networks

Authors: *T. OHNO[1], M. KURODA[2], S. FUKUDA[1], N. MURABE[1], T. HAMADA[1], H. MIYAKE[1], T. MIYASHITA[1], M. NIIDO[1], S. KATO[3], K.-I. INOUE[4], T. HAYASHI[1]; [1]Teikyo University, Sch. of Med., Tokyo, Japan; [2]The Univ. of Tokyo, Tokyo, Japan;

[3]Fukushima Med. Univ., Fukushima-City, Japan; [4]Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Japan

Abstract: The red nucleus (RN) is traditionally divided into the magnocellular and parvocellular part, where existing neurons have been thought to project to the spinal cord (SC) and inferior olive nucleus (IO), respectively. However, neurons of varying size are found in each part, and the rubrospinal pathway were shown to include outputs also from parvocellular neurons indicating that the morphological division of the RN does not by itself completely define the component and functional parts of this nucleus. In this study, we classified the cells of RN into 3 groups by projection targets: rubrospinal (RS), rubro-olivary (RO), and rubro-thalamic (RT) cells. We accomplished this classification by retrograde labeling of their respective targets, SC, IO, and posterior thalamic nucleus (PT). We compared their distribution within the RN, distribution of presynaptic cells, the neurotransmitter phenotypes, and the cell characteristics.First, we examined the intranuclear distribution of 3 groups. By introducing whole brain clearing with 3D analysis using light-sheet microscopy, we obtained the intranuclear 3D atlas of the three-color-labeled origin cells within the same individual. We found that each cell group was widely located along the rostro-caudal axis. Most of the cells were singly labeled.Second, we investigated the distribution maps of the presynaptic cells using retrograde transsynaptic labeling with rabies virus (RV) and the whole brain clearing 3D analysis. Selective expression of RVG (G protein) and TVB (EnvB receptor) in three distinct population was achieved by injecting AAV2retro-Cre into the respective targets and AAV1-Flox-TVB and -RVG into the RN. Injection of EnvB pseudotyped RVΔG (RVG-deficient RV)-GFP thereafter into the RN allows only RV-infected cells expressing RVG and TVB to reach presynaptic cells, enabling mapping of GFP-positive cells. We found difference in distribution pattern between RS and the other two cells.Third, to compare the ratio of GABAergic and Glutamatergic transmitter phenotypes of RO and RT cells, those were shown to possess VGluT2 and VGAT on the axon terminals based on our previous findings, we first employed VGAT-Cre mice to label the inhibitory cells of each population. In contrast to widely studied RS cells, which were all VGluT2-positive, RO and RT cells contained VGAT positive neurons.Fourth, we studied their cell characteristics with whole-cell recordings by optogenetic stimulation. We investigated the characteristics of synaptic inputs from each presynaptic cells. We recorded cortico- or cerebello-rubral EPSCs by stimulating each axon with blue light. The differences were found between RS and other two cells.

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Poster

PSTR396: Cerebellar Interactions With Other Brain Areas and Functions

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR396.23/V4

Topic: F.02. Cerebellum

Title: Learned changes in cerebellar output produce widespread alterations in motor-related dynamics across the brain

Authors: *G. HUGHES;
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Abstract: Skilled motor behavior emerges through learning in distributed brain networks. The cerebellum plays a central role by using sensory prediction errors to rapidly adjust its output, guiding future movements. While cerebellar contributions to local motor refinement are well established, how cerebellar-driven learning reshapes network-wide connectivity and population-level neural dynamics during adaptation remains unclear. To address this, we combined multisite Neuropixels recordings with closed-loop optogenetic stimulation of pontocerebellar inputs during a skilled reaching task in head-fixed mice. This approach allowed us to probe how cerebellar learning dynamically reweights functional influence across the cerebello-thalamo-cortical (CTC) circuit and to assess how these connectivity changes correspond to shifts in population-level neural dynamics. We found that cerebellar perturbation at reach initiation induced rapid kinematic adaptation, consistent with cerebellum-dependent motor learning. Simultaneously, block-specific changes in functional connectivity emerged across the CTC network, with notable modulation of information flow between the cerebellum, motor thalamus, and primary motor cortex. These connectivity changes were accompanied by structured shifts in low-dimensional population neural trajectories, revealing that cerebellar learning not only corrects movement errors but also reorganizes downstream network dynamics to support adaptation. Our results indicate that cerebellar-driven learning flexibly reweights functional influence across distributed circuits, suggesting a hierarchical reorganization of neural dynamics that stabilizes skilled behavior. These findings highlight the cerebellum's broader role in coordinating network-wide adaptation, providing a framework for understanding how local learning events shape global network states and ultimately enable motor skill acquisition and consolidation.

Disclosures: G. Hughes: None.

Poster

PSTR397: Interlimb and Bimanual Control

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR397.01/V5

Topic: F.04. Voluntary Movements

Support: NINDS Grant R01 NS114046

Title: A neural mechanism of handedness for drawing: a left hemisphere motor-premotor system directly supports the dominant hand, and indirectly supports the non-dominant hand

Authors: *N. KAPIL[1], T. KIM[5], S. GASSASS[1], M. D. WHEELOCK[2], R. ZHOU[3], A. R. CARTER[6], I. G. DOBBINS[8], M. MCAVOY[7], Y. WANG[4], B. A. PHILIP[9];

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Abstract: The neural mechanisms of handedness remain largely unknown, especially in the context of natural precision movement with strong lateralization (e.g. drawing). These mechanisms are particularly relevant for patients with chronic unilateral impairment of their dominant right hand (RH) who may need to perform precision movements with their non-dominant left hand (LH). Here, we used functional magnetic resonance imaging (fMRI) to investigate how healthy adults and individuals with peripheral nerve injury (PNI) perform a visually guided drawing task using both hands. We hypothesized that the left superior parietal lobule (SPL) contains a specialization that supports drawing with either hand, i.e. the RH contralaterally and the LH ipsilaterally. We also hypothesized that patients with PNI would use the same neural mechanisms as healthy adults.**Methods:** 34 Right-handed adults (23 healthy controls and 11 with RH PNI) underwent fMRI while performing a precision drawing task, alternating between hands. We performed two parallel fMRI analyses on 12 a priori regions of interest (ROIs): 6 brain areas (SPL, primary motor cortex M1, parietal area 7A, intraparietal sulcus IPS, supplementary motor area, and dorsal premotor cortex) * 2 hemispheres. In the first analysis, we measured task-specific BOLD magnitude in each ROIand modeled it using general linear mixed models with factors chosen from: drawing hand, group, age, sex, and 12 performance variables (the 4-5 top factors, avoiding collinearity). In the second analysis, we measured task-related changes in ROI functional connectivity (FC) via generalized psychophysical interaction.**Results:** LH drawing involved heightened activation and FC in a bilateral motor/premotor network centered on ipsilateral left M1. Specifically, during LH drawing (vs. RH), ipsilateral left M1 had greater activation and greater FC with three areas: contralateral right M1 (reduced activation), contralateral right SMA (reduced), and ipsilateral left PMD (unchanged). We found no effects of patient group.**Conclusions**: These findings suggest that drawing with either hand is supposed by a left-hemisphere M1-PMD network (not our hypothesized left SPL area). This left M1-PMD network acts interhemispherically (directly) during LH drawing and intrahemispherically (indirectly) during RH drawing, which may cause the performance asymmetries associated with handedness. Patients with PNI use the same mechanism as healthy adults, suggesting that following peripheral RH impairment, neuromodulatory interventions may enhance LH performance by targeting this existing mechanism.

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Poster

PSTR397: Interlimb and Bimanual Control

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Program #/Poster #: PSTR397.02/V6

Topic: F.04. Voluntary Movements

Support: DFG LA 1249/2-1

Title: Right hemispheric control of nonverbal gestures in patients with complete callosotomy

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Abstract: *Introduction:* While each hemisphere controls movements of the contralateral hands the production of nonverbal gestures may rely on hemispherically specialized functions. We investigated in patients with complete callosotomy the hypothesis that nonverbal gestures but not hand movements per se rely on hemispherically specialized functions. *Methods:* Gestural expressions were investigated in two patients with complete callosotomy (CC), five patients with partial callosotomy (PC; three patients with anterior callosotomy and two posterior callosotomy), and eleven healthy individuals (C; all right-handed) and in response to tachistoscopically presented pictures of everyday life actions (in the left (LVF) and right visual field (RVF)). The participants were asked to demonstrate with their hands but without speaking what they had seen on the screen. Two independent blind raters evaluated the videotaped hand movements and gestures employing the Neuropsychological Gesture (NEUROGES®) Coding System. *Results:* Over all hand movements categories, patients with CC showed increased contralateral hand movement production in response to presentations to either visual field. Patients with PC showed a lateralization to the right hand (rh). Cognitive complex hand movements showed to be lateralized to the left hand (lh) in patients with CC but to the rh in patients with PC. Patients with CC showed a contralateral response for *spatial relation presentation* gestures. For *pantomime* gestures, patients with CC used either their right or left hands only whereas patients with PC and healthy controls executed *pantomime* gestures with their rh and particularly both hands. Patients with PC did not execute *pantomime* gestures with their left hand. *Discussion:* The present study shows that both hemispheres possess the faculty to produce hand movements and gestures in response to action stimuli. The fact that patients with complete callosotomy showed to produce pantomime gestures with the right and left hand challenges the view that praxis production constitutes a cognitive function of the left hemisphere.

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Poster

PSTR397: Interlimb and Bimanual Control

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Topic: F.04. Voluntary Movements

Support: JSPS KAKENHI 24K20645
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The Cooperative Study Program of National Institute for Physiological Sciences 25NIPS611

Title: Bimanual specific activations in the cerebellar vermis, visual areas, and the parieto-premotor regions: an fMRI study

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Abstract: Mirror therapy has been employed to enhance motor function in individuals who have experienced a stroke. During mirror therapy, a mirror is positioned in the patient's midsagittal plane, thereby reflecting movements of the unaffected side as if it were the affected side. Despite the well-established effectiveness of mirror therapy, the neural mechanism underlying its therapeutic effects remains to be elucidated. Mirror therapy provides visual feedback of bimanual mirror movement, which may enhance bimanual coordination, leading to an improvement in the affected unimanual control. We hypothesized that the overlap of neural substrates between unimanual and bimanual mirror movements causes the effectiveness of mirror therapy. We conducted a 7T fMRI study during the learning of the serial reaction time task (SRTT) to test this overlap. We assumed that the areas activated by the bimanual SRTT more than the sum of the right-hand and left-hand SRTT represent the bimanual coordination-specific function. Thirty healthy right-handed volunteers practiced visually cued unimanual SRTT of random movements or repeating fixed sequences to separately depict the neural substrates of unimanual posture control for chord formation and those of sequence. The identical procedure was performed with bimanual "mirror" mode SRTT. The mirror mode asked participants to press the response buttons simultaneously with homologous fingers on both right and left hands. The behavioral analysis confirmed the implicit sequence learning and chord formation learning. During random movement, we found bimanual-specific constant activation in the cerebellar vermis, bilateral visual cortices, and the right parieto-premotor regions. The chord formation learning effect, neurally represented by the decrement of the task-related activation during random movement, was found bilaterally across the cerebro-cerebellar network during bimanual and right-hand practice. At the same time, it was less prominent and left-lateralized during left-hand practice, indicating that the neural substrates of the right-hand chord learning overlap with those of bimanual mirror-mode chord learning. The sequence learning effect was found in the right parieto-premotor regions. These findings are consistent with the notion that unimanual movement represents a special case of bimanual coordination, with the specific absence of the cerebellar vermis activation, which is specific to the bimanual coordination.

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Poster

PSTR397: Interlimb and Bimanual Control

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Program #/Poster #: PSTR397.04/V8

Topic: F.04. Voluntary Movements

Title: Noninvasive Stimulation of the Corpus Callosum to Modulate Interhemispheric Connectivity and Sensorimotor Performance

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Abstract: The corpus callosum plays a critical role in motor behavior by transferring signals between the two hemispheres, thereby facilitating bimanual coordination during complex sensorimotor tasks. Its role becomes even more important in individuals affected by unilateral stroke, as the intact hemisphere often compensates for lost function. Previous studies have shown that the integrity of the corpus callosum is positively correlated with motor recovery, particularly in tasks that require bimanual coordination. In this study, we investigate whether motor performance can be enhanced through neuromodulation of the corpus callosum. Specifically, we stimulate the posterior midbody of the corpus callosum, the region connecting the bilateral primary motor cortices, using transcranial ultrasound stimulation (TUS), to modulate the interhemispheric connectivity. Two stimulation protocols are applied, with pulse repetition frequencies of 5 and 2000Hz, intended to induce inhibitory and facilitatory effects, respectively. Stimulation effects are assessed by calculating the ratio of interhemispheric inhibition (IHI). Additionally, to evaluate the potential clinical application of this approach, we compare sensorimotor performance before and after the intervention. During the motor task, 15 participants performed bilateral contractions of their first dorsal interosseous muscles to control a cursor's position on the screen, with one hand modulating the horizontal position and the other controlling the vertical position. Our findings indicate that noninvasive stimulation of the corpus callosum can modulate interhemispheric communication, offering a potential therapeutic strategy for improving bimanual coordination in individuals with impaired motor function.

Disclosures: S. Bao: None. **Y. Lin:** None. **Y. Lei:** None.

Poster

PSTR397: Interlimb and Bimanual Control

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Program #/Poster #: PSTR397.05/V9

Topic: F.04. Voluntary Movements

Title: Compensatory strategies affect bimanual function following glove-induced impairment

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Abstract: Successful performance of everyday bimanual tasks relies on asymmetric hand roles, with the dominant hand typically executing more dexterous actions (e.g. grasping) and the non-dominant hand providing a more supporting role (e.g. stabilizing). However, it remains unclear how this hand selection adapts when the dominant limb is impaired. Here, we investigated the impact of impairing the dominant limb on bimanual performance in people without impairments. Fifty-four right-handed young adults completed a *block building task* (BBT), (i) under normal conditions, and (ii) while wearing a Marigold® kitchen glove on their dominant hand. They assembled 8 LEGO® models varying in brick sizes (small or large) and model complexity (6 or 12 bricks). As predicted, participants strongly favoured their dominant limb for the grasping component of the task under normal conditions. However, when wearing a glove on their dominant hand, performance was immediately modified with a significant increase in non-dominant hand use for grasping. Interestingly, this adaptation was selective and strategic, with significantly greater compensation observed where dexterity requirements were more demanding (i.e. for small brick buildings). Together, our data suggest hand selection during bimanual tasks is highly flexible in the face of unimanual impairment. Regardless of established preferences, hand choice appears to include a substantial competitive element, whereby the limb most adept to complete a task at any given time 'wins' the competition for selection. Our data have important implications for understanding how people adapt following injury or disease (e.g. hemiparesis following stroke) and related rehabilitation.

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Poster

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Topic: F.04. Voluntary Movements

Support: NIH NINDS R35NS122333
Medical Science Training Program (MSTP)

Title: Ipsilateral movement representations in human motor cortex

Authors: *K. ADEGEBSAN[1], A. M. EMONDS[2], S. E. PALMER[2], N. G. HATSOPOULOS[2], A. R. SOBINOV[2];
[1]Committee on Computat. Neurosci., [2]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: Motor cortex (MC) is traditionally associated with control of contralateral body movements. However, non-decussating descending fibers suggest a role in coordinating ipsilateral actions, potentially contributing to bimanual manipulation in humans. While ipsilateral arm representations in MC have been documented, representations of ipsilateral hand movements remain largely unexplored. Here, we investigated the presence and nature of ipsilateral finger and wrist movements in MC. We leveraged a clinical trial involving two participants implanted with multielectrode arrays in MC. Participants were visually cued to attempt flexing individual fingers on the left or right hand or combinations of fingers on both hands. We found that the cued ipsilateral digit could be classified with above-chance accuracy based on MC activity during unimanual trials in each subject (83% and 65%). Contrary to our expectations, ipsilateral accuracy was only slightly lower than that for the contralateral digits. However, ipsilateral finger classification accuracy dropped significantly during trials involving both hands. Curiously, during bimanual trials, neural information about the ipsilateral finger emerged earlier than information about the contralateral digit - even when the contralateral cue was presented first. This temporal pattern suggests a potential role for ipsilateral representations in movement planning, while contralateral signals may reflect execution. The results were largely similar for the wrist. Together, these findings reveal robust ipsilateral hand representations in the motor cortex and elucidate the interactions between those representations and contralateral hand control during bimanual movements.

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Poster

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Program #/Poster #: PSTR397.07/V11

Topic: F.04. Voluntary Movements

Title: Effects of Body Weight Support on Limb Kinematics and Coordination During Standing of Near-Walking Infants

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Abstract: Understanding the opportunities for movement and learning that body weight support (BWS) devices may provide is important for designing better motor training programs. Leg

movements, such as kicking and early stepping, have been linked to the development of walking, and facilitating these early on may contribute to an earlier walking onset acquisition for infants with developmental delays, such as those diagnosed with Down syndrome (DS). Additionally, coordinating upper and lower limb movements as infants learn how to stand by themselves independently reflects emerging sensorimotor integration and postural control strategies. This study examined the immediate effects of BWS on limb movements and inter-limb coordination during standing in infants. Eleven typically developing (TD) infants (M=9.9 ±1.2 months) and five infants diagnosed with DS (M=17±3 months) completed two testing sessions within a week, with at least two days of rest between sessions. In each session, infants participated in upright postural activities with and without BWS. Video recordings from the sessions were used for digitization to obtain the 2D position of hands and ankles in every frame. Limb movement was assessed via the average number of Movement Units (MUs), and inter-limb coordination using normalized cross correlation and zero-lag Pearson correlation. To assess differences between the two conditions, non-parametric Wilcoxon signed-rank tests were performed. With BWS assistance, TD infants showed a significant increase in inter-limb coordination ($p<0.001$), as well as leg movement, as measured through the MUs ($p=0.003$). Inter-limb coordination also increased in infants with DS when exposed to BWS ($p=0.019$), whereas a trend toward a greater number of MUs ($p=0.084$) was observed. Our findings demonstrate infants' ability to immediately adapt to changes in biomechanical, environmental, and task-related factors. By manipulating BWS, we altered the lower limbs' weight-bearing demands, which in turn facilitated the re-emergence of spontaneous leg activity. In addition, infants were able to work on the coordination of their upper and lower limbs. This rapid adaptation, observed within only two sessions, suggests a significant learning capacity in TD infants. Infants with DS may need more sessions to display this ability.

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Poster

PSTR397: Interlimb and Bimanual Control

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Topic: F.04. Voluntary Movements

Support: JSPS KAKENHI Grant Number 25K21066

Title: Spatial Modulation of Credit Assignment in Bimanual Motor Learning

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Abstract: The motor system performs complex movements by flexibly coordinating multiple body segments. Tasks such as lifting objects or manipulating tools require the integrated use of

both hands. A key question in motor control is how the system adjusts multiple effectors in response to movement errors both within a trial (feedback response: FBR) and across trials (learning response: LR). Previous studies have shown that, when both hands control a single object, the non-dominant hand tends to be assigned a greater share of the error due to its higher variability, resulting in larger FBR and LR (White & Diedrichsen, 2010). While these findings focused on only effector's properties, how spatial relationships between effectors and the manipulated object systematically influence credit assignment remains unclear, though our previous work suggests that spatial congruency in sensory feedback may affect this process (Kasuga & Nozaki, 2011). Here, we examine how spatial factors shape error assignment during bimanual motor learning.Participants held two handles positioned at ±10 cm from the body midline and performed a reaching task by moving a cursor toward a target located 15 cm ahead from the starting position. The task was performed under three conditions: unimanual-right, uni-left, and bimanual. The starting position (and corresponding target) was presented at one of seven possible locations relative to the midline (±20, ±10, ±5, and 0cm). The cursor position was systematically changed according to the starting position. Each trial set consisted of four trials: a baseline, a perturbation, a probe, and a washout trial. Within the set, the movement condition (uni- or bimanual) and the target position were held constant. In the perturbation trials, the cursor was laterally shifted by ±1.5 cm at 3 cm from the starting position. FBRs and LRs were measured as lateral forces exerted against a force channel during the perturbation and the subsequent probe trial, respectively. Participants were instructed not to use any explicit strategy to correct movements.This design enabled us to examine how FBR and LR were influenced by the spatial position of the cursor under uni- and bimanual conditions. In the bimanual condition, both responses of a given hand were modulated by the cursor position: the responses increased as the cursor approached the hand. However, in unimanual conditions, the response remained nearly constant regardless of cursor position, indicating that spatial modulation observed in the bimanual condition was absent. These results demonstrate that the motor system incorporates spatial information into the computation of credit assignment during bimanual movements.

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Poster

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Title: White Matter Integrity and Bimanual Coordination in Hemiplegic Cerebral Palsy: A Robotic Assessment Study

Authors: *H. D. GASCON[1], O. MARTINIE[2], M. DESCOTEAUX[4], C. MERCIER[5], M. T. ROBERT[3];
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Abstract: Bimanual actions, which involve the coordinated use of both hands in symmetric or asymmetric patterns, are supported in part by the corticospinal tract (CST) for motor execution and the corpus callosum (CC) for interlimb communication. In children with unilateral cerebral palsy (CP), disruptions in the structural integrity of the CST and CC have been associated with reduced performance in bimanual motor tasks. However, these pathways are part of a broader neural network, and their specific contributions to bimanual motor control remain to be fully understood. Seventeen children with unilateral CP (mean age 10.7 ± 2.1 years, 7 females) with mild to moderate motor impairments were recruited. Participants underwent diffusion magnetic resonance imaging (MRI: 3-Tesla Phillips) to acquire anatomical and diffusion MRI metrics. Participants completed two behavioral bimanual tasks (Object Hit [symmetric] and Ball on Bar [asymmetric]) using the Kinarm Exoskeleton Robot. The following behavioral parameters were extracted: task success, spatiotemporal parameters (i.e., hands speed and time to reach a target), and bimanual coordination. CC tractography and segmentation lead to two regions of interest (i.e., parietal and pre-postcentral segments). Tractography of both the CST and CC yielded five diffusion metrics: fractional anisotropy, mean diffusivity, tract volume, number of fiber orientations, and neurite density index. The CC metrics showed multiple correlations (Spearman) with spatiotemporal parameter for both bimanual tasks (p-values ≤ 0.05). In contrast, most of the CST metrics were only correlated with spatiotemporal features during the asymmetric task (p-values ≤ 0.05). Parietal segment of the CC was correlated with task success (p-values ≤ 0.03). After adjusting for age with partial correlation, neurite density index in the pre-postcentral segment ($r = 0.575$, $p = 0.04$) and fractional anisotropy in the parietal segment ($r = 0.578$, $p = 0.04$) were correlated with the bimanual coordination parameter during the asymmetric task only. These findings highlight the important role of the corpus callosum in supporting bimanual motor function in children with CP, particularly during asymmetric tasks. Compared to the CST, CC integrity shows a more consistent association with successful bimanual coordination.

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Poster

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Topic: F.04. Voluntary Movements

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Title: Hold On or Let Go: Intention and fluctuation in bimanual coordination

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Abstract: Life's biggest dilemmas can be summed up as a tension between holding on and letting go. Yet, basic insights into how coordinative structures or functional synergies stabilize or evolve have come from a seemingly simple task: wiggling one's fingers to and fro to the beat of a metronome1. As the metronome pace increases, one coordinative pattern becomes unstable and switches spontaneously to another. Such transitions are typically preceded by critical fluctuations, a predicted feature of self-organization in complex, dynamical systems. Here we address the nature and source of these fluctuations, usually assumed to be: 1) random; 2) of external origin; and 3) of fixed magnitude. We performed an experiment in which participants were instructed to oscillate their fingers in either an in-phase or anti-phase pattern in time with a metronome and instructed them to either "hold-on" or "let-go" should they feel the pattern change. We found that, as the metronome frequency was increased from 1 to 3.75 Hz, the spatial and temporal characteristics of the fluctuations in the relative phase between the fingers were significantly altered by a participant's intention to "hold on" or "let go" of the instructed pattern. Furthermore, analysis of the coordination between the hands and the metronome revealed rich dynamics in which intention to stabilize the coordination between the hands alternates fluidly with the requirement to coordinate the hands and the environment. Our results establish a new approach to quantify the 'mind force' of intention on coordination via fluctuation analysis and hint quite strongly that the underlying dynamics is metastable, exhibiting simultaneous tendencies to hold on and let go. Critically, these results beg reconsideration of the nature and source of coordinative fluctuations in animate behavior and their relation to intentionality (including cognate terms such as 'purpose' and 'agency'). 1 With respect to deciding to move one's finger... "It's as if we're coming to understand the puppet and the strings, but we know nothing about the puppeteer. That remains as much a mystery as it has been since classical Greece" (Chomsky & Moro, 2022, p.60).

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Poster

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Topic: F.04. Voluntary Movements

Support: DOD AFRL FA9550-24-1-0208

Title: Locomotor assessment of obstacle negotiation during split-belt treadmill walking in virtual reality

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Abstract: Impaired visuomotor control of foot placement is a significant contributor to falls, which limits mobility and overall health and well-being. Our study rationale was to integrate an immersive virtual reality (VR) task with precise limb placement on a self-paced split-belt treadmill. We expected that visuomotor planning and execution would be appropriately engaged in obstacle negotiation. Participants were instrumented with motion-tracking sensors of distal limb segments, enabling the approximate kinematic tracking of the entire body in real-time. The custom pipeline using SteamVR-Unity-Matlab bidirectional communications allowed us to develop an environment where various obstacles appeared over two steps in advance of the subject's current position. The limb end-point foot kinematics adjusted consistently to the variation in obstacle height and orientation, validating the successful planning and execution of stepping. However, while the end-point limb trajectories were not "colliding" with the virtual objects, the stepping height was not optimal as participants overestimated the expected clearance. The task allowed for both leading and trailing types of obstacle negotiation, indicating that the reaction time was appropriate and similar to the natural time course. Overall, the VR task was successfully executed and can be further developed to challenge steering while walking in a cluttered space. The safety and opportunities for integrating consistent biomechanical assessment, as well as other wearable technologies, are promising foundations for a new type of locomotor rehabilitation.

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Poster

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Topic: F.04. Voluntary Movements

Title: Reflex Responses to Unilateral Perturbations During Bimanual Reaching

Authors: V. KALEPU, ***F. A. KAGERER**;
Michigan State Univ., East Lansing, MI

Abstract: Bimanual coordination plays a critical role in many everyday tasks, yet how each limb responds to mechanical disturbances under different task constraints remains poorly understood. The purpose of this study was to determine whether reflex responses during a bimanual reaching task were modulated by functional asymmetry of the motor system. Using the Kinarm Endpoint Lab, we had two tasks: In the *Common Cursor* (CC) condition, right-handed participants (n = 14) controlled a shared cursor using equal contributions from both hands to reach a target of 15 cm above the start position. In 20 out of 110 trials, an outward mechanical perturbation pulse (50N for 50ms) was randomly applied to either hand during 100ms after movement initiation. In the *Elastic Bar* (EB) condition, each hand controlled one end of a connecting virtual bar, and participants were instructed to maintain a target bar length while reaching across the same distance as in the CC condition. Perturbations were again randomly applied unilaterally. In both tasks, we recorded EMG from the anterior and posterior deltoid muscle bilaterally.Kinematic analysis revealed that in the CC condition, both the perturbed and unperturbed arms reacted in opposition to the perturbation, with highly symmetrical responses between left and right arms. In contrast, the EB condition showed reduced resistance from the unperturbed hand, reflecting behavior predicted from an optimal feedback control perspective to compensate for changes in bar length. The kinematic data also indicated small lateral asymmetries - specifically, the right unperturbed hand exhibited a quicker opposing response than the left. EMG analysis showed a short-latency response (~20-30ms) in the anterior deltoid of the perturbed arm, with slightly greater amplitude on the non-dominant left side in the CC condition. Notably, the posterior deltoid on both perturbed and unperturbed sides exhibited near-identical reflex onset latencies (50-70ms) and amplitudes, suggesting similar bilateral antagonist engagement during unilateral perturbations, likely related to postural stabilization. The anterior deltoid of the unperturbed arm showed longer-latency responses (100-120ms) in both conditions.

Disclosures: V. Kalepu: None. **F.A. Kagerer:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.01/W6

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Integrative Approaches to Explore Cellular Interactions in Neural Circuits T32
NIH NINDS U19
NIH NICHD R01
Global STEAM

Title: Mouse midwives: behavioral mechanisms that greatly improve maternal-infant survival during difficult parturition

Authors: *V. J. IVAN[1], L. SCHUSTER[3], D. GILLY SUAREZ[2], S. B. WINOKUR[1], R. J. HENDERSON[4], A. C. MAR[5], R. C. FROEMKE[6];
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Abstract: Oxytocin is an ancient neuropeptide that regulates mammalian reproduction and parental care including uterine contractions for labor (Gimpl & Fahrenholz 2001, Dulac et al. 2014, Froemke & Young 2021). Mice lacking oxytocin receptors (OXTR KOs) do not have uterine contractions (Takayanagi et al. 2005) but can still reproduce. It remains unclear how disrupted oxytocin signaling affects reproductive behavior (Berendzen et al. 2022). Given rising rates of human maternal mortality (Hoyert 2023), it is important to determine physiological and behavioral factors associated with successful childbirth. We built a system for 24/7 continuous multi-camera monitoring of mouse family life (Schuster et al. bioRxiv 2025). We compared maternal and pup survival outcomes of first-time pregnant OXTR KO females co-housed with lactating experienced wild-type dams vs outcomes of singly-housed pregnant OXTR KO females. When co-housed, experienced wild-type dams actively assisted in labor, performing 'midwife' behaviors such as extracting stuck pups with their mouth or paws, cleaning newborn pups of amniotic sacs, and consuming placenta. Solitary OXTR KO dams had low survival rates, with only 1 out of 7 dams surviving labor and 0% pup survival. Assistance from wild-type midwives resulted in a remarkable 9/10 survival rate for OXTR KO dams and a 90.2% pup survival rate. Co-housing with the paternal male, a virgin female, or an OXTR KO female was less effective with ~50% of dams surviving but 0% pup survival. Labor duration of OXTR KO females was reduced when co-housed with lactating dams (5.8±2.4 min/pup) vs cohousing with paternal males (68.8±31.1 min/pup) or nulliparous females (25.3±5.5 min/pup). Lactating dam midwives spent more time attending to the pregnant dam (47.0±6.3% total time of labor) compared to paternal males (3.7±1.7%) or nulliparous females (10.3±6.2%). Frame-by-frame behavioral analysis revealed temporal synchronization of companion interactions during labor events. Pup extraction more often transitioned to pup cleaning when dams were cohoused with lactating females, whereas paternal males transitioned from inactivity to mounting behaviors, and nulliparous females transitioned more from grooming to abdominal pressing. We have also observed labor assistance for wild-type pregnant dams. Our findings underscore the critical role of experienced maternal support in childbirth, highlighting the significant benefits of midwife-like behaviors for maternal and offspring survival during dystocia or extended labor.

Disclosures: V.J. Ivan: None. **L. Schuster:** None. **D. Gilly Suarez:** None. **S.B. Winokur:** None. **R.J. Henderson:** None. **A.C. Mar:** None. **R.C. Froemke:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.02/W7

Topic: G.02. Neuroendrocrine Processes and Behavior

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DKOP-23 Doctoral Excellence Program
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MTA NAP2022-I-3/2022 (NAP 3)

Title: The lateral septal calbindin neurons promote pup-caring behavior in rodents

Authors: *G. PUSKA[1,2], V. SZENDI[2], M. EGYED[2], A. DOBOLYI[3];
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Abstract: In mammals, critical changes during pregnancy and the postpartum period ensure the survival of the offspring, who are unable to develop and thrive independently in the early postnatal period. During this period, mothers receive various sensory cues from their pups, which are processed by a complex neural circuit. The lateral septum (LS), a forebrain area, is part of this network. The lesion of the area has been previously shown to induce maternal dysfunction, however, the role of the LS in maternal care has not yet been established. Furthermore, emerging evidence suggests that a significant change that occurs after parturition is the elevated expression of a neuropeptide induced in mothers, parathyroid hormone 2 (PTH2). The distribution of PTH2+ terminals in the ventral subdivision of the LS (LSv) also suggests that this area may serve as a site of maternal actions. To gain a profound understanding of its function, advanced histological techniques were applied and functional experiments performed. Firstly, the objective was to ascertain the impact of cue modality from the pups on the septal activation in the context of pup deprivation. The highest degree of LSv activation was attributed to direct contact with the pups, which included suckling. Consequently, we examined the inputs of the LSv that may mediate the somatosensory modality of suckling. The retrograde tract tracing method was employed, revealing that neurons within the posterior intralaminar thalamic nucleus (PIL) project to LSv neurons. Moreover, PIL neurons have been demonstrated to express suckling-induced PTH2, which we have shown to act on activated LSv neurons by forming a synaptic connection. Additionally, it was elucidated that these neurons are GABAergic calbindin (Cb) neurons. Consequently, we initiated an investigation into the function of LSv Cb neurons in maternal care. A transgenic mouse line expressing Cre-recombinase in its calbindin neurons was utilized. The behavioral analysis indicated that the maternal licking behavior exhibited by the mothers was diminished when their LSv Cb neurons were subjected to chemogenetic inhibition. Furthermore, it was determined that the septal neurons activated by pups send their most prominent projection to the medial preoptic area (MPOA), the primary regulatory site of maternal care, which also receives a high number of PTH2+ terminals. Therefore, we performed double retrograde injections, which revealed that the same neurons within the PIL can project to both LSv and MPOA. In summary, the available data suggest that the LSv and MPOA form a subcircuit that regulates maternal care, which is potentially governed by thalamic PTH2 input.

Disclosures: G. Puska: None. **V. Szendi:** None. **M. Egyed:** None. **A. Dobolyi:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.03/W8

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Effect of paternal behavior on anxiety, spatial memory, and pup care in male rats

Authors: *A. M. GARCIA, J. M. IBARRA HERNÁNDEZ, A. PALOMINO-CRUZ, M. PERALES-QUINTANA, A. DAMIÁN LÓPEZ, A. GONZÁLEZ;
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Abstract: Parental behavior has been extensively studied in females, showing benefits such as improved memory and reduced stress. In male rats, however, the effects of fatherhood on cognition and emotional behavior remain poorly understood. Our objective was to evaluate memory, anxiety, and paternal behavior in male Wistar rats exposed to varying numbers of litters.Male Wistar rats were divided into three groups based on the number of litters they were exposed to: G1 (one litter), G2 (two litters), and G3 (three litters). Paternal behaviors were analyzed in the presence and absence of the female for 10 minutes on postnatal days (PND) 1, 3, 5, 8, 10, 12, 15, 17, and 19. The following behaviors were quantified: latency to first paternal behavior, sniffing, licking (body and anogenital), huddling (pup covering), grooming, defecation, and stepping over pups. Anxiety was assessed using the Elevated Plus Maze (EPM), and short-term memory (STM) and long-term memory (LTM) were evaluated using the Barnes maze.In the Barnes maze, training latency decreased by up to 30% in males that cared for litters, showing improved LTM (latency: 8 ± 1 s) compared to non-caregivers (30 ± 5 s). G2 males showed a significant increase in time spent in the closed arms of the EPM (290 ± 5 s), indicating elevated anxiety. Likewise, latency to initiate paternal behavior increased in G3 males on PND 5 and PND 10 compared to G1, and huddling (pup covering) behavior also increased in G3 regardless of female presence.In conclusion, prolonged exposure to litters promotes the development of active paternal behaviors, which are associated with increased anxiety and enhanced cognitive performance. These findings suggest that fatherhood in male rats not only modulates pup-directed behavior but also induces adaptive changes in emotional and cognitive processing, as previously reported in females.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Program #/Poster #: PSTR398.04/W9

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant 2346296

Title: Changes in neural expression of vitellogenin associated with parental care in burying beetles

Authors: L. E. CHAHINE, M. M. KERMAGI, *S. C. PANAITOF;
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Abstract: Burying beetles, *Nicrophorus orbicollis*, have extended biparental care of young. To breed, a male-female pair of beetles bury and prepare a small vertebrate carcass as food for their young. Upon hatching, larvae depend on parental regurgitations of food to survive. The neurophysiology of this remarkable caregiving behavior is however poorly understood. Intriguingly, several candidate molecules known to be broadly involved in the regulation of insect reproductive physiology appear to have been co-opted for a social role in *N. orbicollis*, including in the coordination of parental activities and the intensity of care. These include Juvenile Hormone and the egg yolk protein precursor vitellogenin (Vg) that it upregulates. In previous work we found that *vg1* and *vg2* transcripts decreased in the fat body of breeding females around the time larvae hatched, compared to prebreeding animals whose *vg* levels were measured at several timepoints during the reproductive maturation period. In this study we measured *vg1* and *vg2* transcript abundance in the head (brain) tissue of parental females after 24 hours of care, corresponding to peak intensity of parental feeding rates, and compared these to those of nonbreeding controls. Relative gene expression of both *vg1* (0.20 ± 0.09, mean ± SE) and *vg2* (0.27 ± 0.13) significantly decreased ($p < 0.001$) in parental females compared to controls (1.07 ± 0.16 and 1.04 ± 0.11, respectively). Moreover, transcript levels of *vg1* and *vg2* were significantly positively correlated in both parental ($R^2 = 0.97$, $p<0.001$) and control ($R^2 = 0.87$, $p<0.001$) females. These results are consistent with previous findings of significant reduction in *vg* expression during active parental care in the sister species, *N. vespilloides*, and consolidate the association between reproductive stage-specific changes in *vg* expression patterns and the expression of parental behavior in *Nicrophorus* spp. Taken together, these findings complement insights from social insects like the honey bees where Vg appears to have acquired non-reproductive roles related to behavioral maturation and caste development, as well as similarly provide additional evidence for a social function of Vg in the regulation of reproduction in burying beetles.

Disclosures: L.E. Chahine: None. **M.M. Kermagi:** None. **S.C. Panaitof:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Program #/Poster #: PSTR398.05/W10

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01AG078758
NIH Grant R01AG062716
NIH Grant T32DA018926

Title: Transcriptomic changes in the parental circuit and cross-species analysis of aged California mouse breeders

Authors: *K. A. CALLAHAN[1,2], B. C. TRAINOR[3], L. K. FONKEN[2,1];
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Abstract: Parenting in advanced age is correlated with higher risk of adverse offspring outcomes, such as neurodevelopmental and metabolic disorders. One possible explanation for this association is the effect of inflammaging, or age-associated chronic inflammation, on the brain. Inflammaging can lead to neurodegeneration and cell death, which may alter the function of the parental circuit and subsequent parental behaviors. We investigated this using *Peromyscus californicus* (California mouse), a monogamous, biparental rodent species that can live up to 5.5 years and remains fertile well into advanced age. We compared gene expression between adult and aged breeders and hypothesized that aged breeders would show upregulation of pro-inflammatory genes in brain regions involved in cognitive aging and parental behaviors. Tissue was collected from the dorsal hippocampus, medial preoptic area, and medial amygdala of adult (N=8, mean age=0.7 yr) and aged (N=7, mean age=3.3 yr) male and female breeders for differential gene expression analysis. Across all regions, aged females had 1,582 DEGs (802 upregulated), while aged males had 2,236 DEGs (1,090 upregulated) when compared to adult conspecifics. Aged breeders showed upregulation of some pro-inflammatory genes, such as *Icam1*, *Irak3*, and *Tlr3*. These inflammatory changes appeared more modest than expected, and did not include the canonical inflammaging-associated genes seen in other model rodent species such as mice and rats. To assess how aging differentially affects gene expression in California mice versus other rodent species, we performed cross-species Rank Rank Hypergeometric Overlap analysis. There was overall discordance in which genes change expression with aging between California mice and C57BL/6 mice, further supporting a discrepancy in inflammatory gene expression. This may suggest that since California mice exhibit delayed maturation, have longer lifespans, and continually reproduce in advanced age, they are evolutionarily protected against the negative effects of aging like chronic neuroinflammation.

Disclosures: K.A. Callahan: None. **B.C. Trainor:** None. **L.K. Fonken:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Program #/Poster #: PSTR398.06/W11

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: SECIHTI national scholarship 1159644

Title: Effect of the hypercaloric diet during pregnancy and lactation on the sexual behavior, prostatic histology and brain dimorphic areas of adult male offspring

Authors: *D. RENDÓN CORIA[1], G. A. CORIA-AVILA[2], M. HERNANDEZ[3], G. E. ARANDA-ABREU[5], R. TOLEDO-CARDENAS[1], J. MANZO[4], D. HERRERA-COVARRUBIAS[1];
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Abstract: Given the current prevalence of high-calorie food consumption it is important to consider the long-term effects of hypercaloric diet during critical periods of brain development and on motivated behavior of the offspring, particularly during the transition into adulthood. Accordingly, we explored the effect of a hypercaloric diet during pregnancy and lactation on the sexual behavior, brain dimorphism and prostatic histology of adult male offspring. Two groups of Wistar females were formed (n=5): 1) fed with cafeteria-type hypercaloric diet (HD, with +80% increase in calorie intake) and 2) control that received standard laboratory feed (SD). Both groups received their diet throughout and 8-week period that includen 2 weeks prior to gestation, 3 weeks during gestation and 3 weeks of lactation. On PND90 we assessed sexual behavior and one day later the brains were removed and stained with Nissl dye and the prostates were stained with hematoxylin and eosin dye. The areas of the sexually dimorphic nucleus of the preoptic area, ventromedial nucleus of the hypothalamus and medial amygdala were measured as well as the alveolar area and the epithelial height of the dorsolateral (DLP) and ventral prostate (VP). Pups from HD mothers showed a higher frequency of ejaculations and a shorter latency to ejaculation, as well as a decrease in the area of the sexually dimorphic nucleus and amygdala. A decrease in the alveolar area of the DLP and a decrease in the epithelial height of the VP were also observed. Prostate histology showed metaplasia in the case of DLP of offsprinf of HD mothers, as well as dysplasia in the VP. The group of rats from SD mothers showed normal tissue when they were sexual naïve, but hypertrophy if sexually experienced. We discuss how this type of diet may affect the organization of brain development in the offspring.

Disclosures: D. Rendón Coria: None. **G.A. Coria-Avila:** None. **M. Hernandez:** None. **G.E. Aranda-Abreu:** None. **R. Toledo-Cardenas:** None. **J. Manzo:** None. **D. Herrera-Covarrubias:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: PSTR398.07/Web Only

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NICHD Grant HD112721

Title: Microglia modulation alleviates depression-related deficits in maternal behavior and affect in Wistar-Kyoto mother rats.

Authors: *M. KRASAKOVA[1], K. COPELAS[2], L. ABRAHAM[2], A. CULLEN[2], M. PEREIRA[2];
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Abstract: Postpartum depression is a serious global public health problem, characterized by significant disturbances in maternal affect and parenting, with tragic consequences for both mother and child. Emerging evidence implicates dysregulation of microglia, the brain's resident immune cells, as a contributing factor in the pathophysiology of depression. The present study aimed to examine whether microglia dysregulation within key components of the maternal neural circuitry, including the medial preoptic area (mPOA), contributes to depression-related deficits in maternal affect and behavior. To this aim, we employed the well-validated Wistar-Kyoto (WKY) animal model of depression and ultrasonic vocalization (USVs), which serve as behavioral indicators of affective states, to examine the effects of minocycline, a microglial modulator, in ameliorating deficits in maternal affect and behaviors in WKY mothers. WKY and control Sprague-Dawley (SD) mothers were evaluated for their maternal behavior following daily systemic treatment with minocycline (1.0 or 10 mg/kg) or vehicle from gestation day 10 to postpartum day 7. Results show that WKY mothers exhibited reduced positive affect and impaired maternal behavior compared to controls, which were negatively correlated with altered microglia morphology, specifically larger territory areas and more complex branching in the mPOA. Treatment with 1.0 mg/kg minocycline shifted microglial morphology and significantly ameliorated deficits in maternal behavior and affect in WKY mothers to levels comparable to SD controls. In contrast, the 10 mg/kg dose exacerbated behavioral deficits and was associated with a higher proportion of hyper-ramified microglia. These findings demonstrate that microglia modulation can alleviate depression-related deficits in maternal behavior and affect, and suggest a mechanistic link between postpartum microglial adaptations in the mPOA and healthy parenting.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: PSTR398.08/W12

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: Universidad Nacional de Colombia. HERMES 60437
Universidad de los Andes

Title: Transgenerational effects on maternal behavior due maternal separation during breastfeeding

Authors: *Z. DUENAS[1], L. MÁRQUEZ[2], L. A. LEÓN[3], F. CÁRDENAS[4];
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Abstract: Maternal separation during breastfeeding (MSDB) is a chronic stress factor that has effects on the mother and her offspring. In fact, stress during early life, increases the risk of suffering several psychopathologies such as major depression, borderline personality disorder, anxiety and substance abuse. Additionally, some studies show that patients who have suffered abuse at early age, develop behavioral disorders promptly and are characterized because the symptoms are severe. On the other hand, it has been shown that maternal behavior can modulate the deleterious effects of stress, so, it has been reported that the offspring of negligent mothers in the care of their offspring, show alterations in the response to stress in adulthood. In this work, we evaluated the intergenerational transmission of changes in maternal behavior caused by separation during breastfeeding. These modifications can be behavioral and include maternal behavior, specifically, behaviors such as suckling, licking and pup's retrieval. The findings of this study show that the behaviors of active suckling, licking, construction of the nest and pup's retrieval are affected due to stress caused by maternal separation. These effects altered maternal behavior in the next generations (F2 and F3), although these have not received the stress stimulus directly. The results obtained suggest the occurrence of epigenetic modifications in genes related to the expression of maternal behavior such as receptors for oxytocin, estrogens and / or glucocorticoids.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Program #/Poster #: PSTR398.09/W13

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIMH R01 MH121829

Title: Molecular characterization of oxytocin neurons in the BNST

Authors: *S. ARCHDEACON[1], A. CHRISMAN[2], A. SERNA GODOY[3], B. C. TRAINOR[4];
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Abstract: Oxytocin is an important neuropeptide that modulates social and affective behavior. Oxytocin neurons have been studied primarily in the hypothalamus, but populations of this cell type also exist in the bed nucleus of the stria terminalis (BNST). It is unknown whether these cells share similar molecular signatures to hypothalamic *Oxt*-expressing neurons. To explore this, we performed single nucleus RNA sequencing on BNST samples from 24 California mice (*Peromyscus californicus*) (12 males, 12 females; 3-6 months old). Sequencing data from ~250,000 nuclei were analyzed using the Seurat package in R, and 283 cells were identified as *Oxt* neurons based on *Oxt* and *Snap25* coexpression. This is consistent with prior findings that *Oxt* neurons make up a small percentage of BNST cells. More *Oxt* neurons were also found in males than females, which is consistent with larger BNST size in males. Unsupervised clustering of this subset (resolution = 0.3) identified three distinct neuron clusters. The cluster with the highest *Oxt* expression also shows the highest *Foxp1* expression, which is of interest because *Foxp1* has been identified as a marker for magnocellular *Oxt* neurons in the hypothalamus. The cluster with the lowest *Oxt* expression coexpresses *Adarb2*, a transcript that has been identified as a marker for hypothalamic parvocellular *Oxt* neurons. These data suggest that as in the hypothalamus, different subtypes of *Oxt* neurons exist in the BNST. Future analyses will investigate how these clusters align with putative magnocellular and parvocellular neurons in the hypothalamus, and whether sex differences in cell type distribution exist.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Program #/Poster #: PSTR398.10/W14

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NSF AAH5162
ABS Research Grant

Title: Division of Labor: Impacts of Context, Reproductive Status, Oxytocin, and Estradiol in California Mice

Authors: *C. L. MALONE[1], C. A. MARLER[2];
[1]Psychology, Univ. of Wisconsin- Madison, Madison, WI; [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Oxytocin (OXT) and Estradiol (E2) signaling are known to impact coordinated, close-range behaviors (e.g. following, contact, huddling) in rodents. However, it is unknown if OXT or E2 impacts spatially-separated coordinated behaviors. The biparental, monogamous California mouse (Peromyscus californicus) coordinates spatially-separated tasks to accomplish spatially-separated goals such as resource acquisition, territorial defense, and parental care. Using this

unique species, we investigated how division of labor changes across the social life span and whether OXT and/or E2 can alter division of labor in the primiparous stage. We manipulated both systems via intranasal or intraperitoneal injections of an agonist, antagonist, or vehicle after the first litter of pups was born. Behavioral analyses of the pairs during their pre-pair, post-pair, and primiparous stages in a nonsocial (foraging) and social (territory intrusion) challenge were conducted. Our early results indicate that during social challenges, mice use more sex-delineated roles, with males spending time near the intruder and females spending time at the nest. In contrast, nonsocial challenges are divided more equitably between the sexes. Division of labor also increases as time demands increase, such as during the primiparous life stage. Under the influence of OXT antagonists, division of labor tactics were disrupted, as evidenced by an increase in time spent foraging together with neglect of the nest. These results indicate a new role of OXT signaling in the coordination of spatially-separated tasks, not just proximal synchrony.

Disclosures: C.L. Malone: None. **C.A. Marler:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 1RO1DA052386
T32 Train@Wayne award (to LMR) DA060139

Title: Adverse offspring outcomes correlate with opioid induced maternal care deficits which may be mitigated by postpartum oxytocin treatment.

Authors: J. DURAN[1], E. R. WALSH[1], L. M. RICHARDSON[1], A. MYERS[1], S. A. PERRINE[2], S. E. BOWEN[1], *S. BRUMMELTE[1];
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Abstract: Many opioid-dependent pregnant women are prescribed medications for opioid use disorder (MOUDs), such as buprenorphine (BUP). BUP is a semi-synthetic opioid with a unique mechanism of action (partial mu-agonist/kappa antagonist) that produces preferable outcomes for exposed infants as compared to methadone or illicit opioid exposure. However, BUP's effects on the maternal brain, behavior, and physiology are not well understood. The current study used a translational rodent model to investigate how transitioning from an opioid of abuse (i.e., morphine) to BUP during gestation affects maternal care and physiology and offspring outcomes compared to continued use of morphine, or BUP from preconception through early postpartum. Dams were assigned to one of five experimental groups: BUP continued (1mg/kg, s.c.), morphine continued (3 - 10 mg/kg, s.c.), morphine to BUP (3 - 5 mg/kg morphine until Gestational Day (GD) 5, then 1.0 mg/kg BUP; s.c.), morphine to vehicle (3 - 5 mg/kg morphine

until GD 5, then 1.0 ml/kg saline; s.c.), or saline continued (1.0 ml/kg, s.c.). BUP exposed dams exhibited less pup-directed behavior compared to controls. Offspring born to BUP exposed dams had decreased body weights and length, reduced milk bands, and increased withdrawal symptoms and mortality as compared to controls. Importantly, these offspring outcomes were correlated with the level of maternal care received, indicating that, in part, adverse effects result from behavioral deficits. Notably, opioid exposed offspring had altered norepinephrine concentrations in the prefrontal cortex and striatum compared to controls, however these changes were not correlated with maternal care suggesting that some effects are directly related to in-utero opioid exposure. Further, preliminary data from a follow-up study investigating the effectiveness of postpartum oxytocin administration suggests that a single dose of intranasal oxytocin (4 IU/kg) can rescue maternal care deficits and subsequent offspring outcomes highlighting a potential intervention strategy. Overall, further investigation is warranted to fully understand how BUP uniquely influences the maternal brain, thus altering behavior and subsequently impacting offspring outcomes.

Disclosures: J. Duran: None. **E.R. Walsh:** None. **L.M. Richardson:** None. **A. Myers:** None. **S.A. Perrine:** None. **S.E. Bowen:** None. **S. Brummelte:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.12/X1

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NSF IOS-2146058

Title: Effects of transdermal corticosterone administration on peripheral hormone levels and partner interactions in dyeing poison frogs (Dendrobates tinctorius)

Authors: *M. C. ACOSTA, L. TAO, E. K. FISCHER;
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Abstract: Glucocorticoids (GCs) are recognized for their role in regulating stress responses and influencing social behaviors across taxa. In dyeing poison frogs (*Dendrobates tinctorius*), changes in GCs are associated with stress, developmental transitions, and parental care. Previous studies demonstrated that breeding pairs in another poison frog species can physiologically state-match their GC levels, however, the importance of behavioral interactions in this phenomenon is unknown. To determine whether GCs can actively shape social dynamics and whether post-treatment social interactions contribute to shared physiological states, we investigate the effects of transdermal corticosterone administration on male hormone levels, untreated female partner hormone levels, and pair interactions. Male frogs received either a low (5 µg) or high (10 µg) dose of corticosterone and a vehicle control, using a randomized, counterbalanced within-subjects design. Each male was tested across two treatment days with a rest day in between.

Following each treatment, males were reunited with their female partners, and pair interactions were recorded for one hour. Water-borne hormone levels were sampled from both sexes one hour before drug administration, as well as 1 hour and 4.5 hours post-treatment. Social behaviors were quantified, including duration of joint sheltering, dyadic physical proximity, exploratory behavior, and conspecific approach frequency. We predict that corticosterone-treated males will exhibit increased circulating corticosterone levels, and that untreated females paired with treated males will show elevated corticosterone levels compared to females paired with control males, consistent with physiological state-matching. Preliminary analyses indicate that corticosterone treatment may influence behavior in a dose-dependent manner, suggesting a potential behavioral mechanism for hormonal coordination between partners. By establishing an effective method for manipulating GCs in *D. tinctorius*, this study provides a critical step toward understanding the causal role of these hormones in social regulation in amphibians.

Disclosures: M.C. Acosta: None. **L. Tao:** None. **E.K. Fischer:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.13/X2

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant 2310626

Title: Steroidal mechanisms of group dynamics in spiny mice (Acomys dimidiatus)

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Abstract: Spiny mice (*Acomys dimidiatus*) are highly social rodents that live in large groups in the wild. In the lab, they exhibit little aggression and are prosocial with conspecifics regardless of age, sex, novelty/familiarity, or kinship, positioning this species as an ideal model for examining neuroendocrine underpinnings of group dynamics. Yet, little is known about the social dynamics of large groups of spiny mice. Moreover, despite extensive literature addressing the role of steroid hormones in avian grouping behaviors, there is a lack of research examining mechanisms of group dynamics in mammals. To elucidate the group dynamics of spiny mice and explore the role of steroid hormones in group interactions, 5 groups of 7 spiny mice of mixed genetic relation (N_{female}=4, N_{male}=3) were formed at weaning and allowed to cohabitate for 40 days. Once adults, groups were placed in a large test chamber with enrichment for 5 hours/day, for 4 consecutive days. Plasma and brain tissue were collected after testing. Plasma was processed via enzyme-linked immunosorbent assays to analyze circulating testosterone, whereas immunohistochemistry was used to label estrogen and androgen receptors in brain tissue. Behavioral recordings were time sampled, generating around 240 timestamps per individual for affiliative, aggressive, and non-overt behaviors. Metrics obtained through social network

analysis (SNA) were used to ascertain the prestige (degree) of individuals within the network, and how connected spiny mice are to conspecifics in their group (eigenvector centrality). Preliminary results from 2 groups show that spiny mice predominantly engage in affiliative behaviors. We observed few sex differences; however, only males (1 in each group) exhibited chasing, which was directed at both males and females. Males and females exhibited similar frequencies of dominance (displacement of conspecifics). Plasma testosterone positively correlated with a combined frequency of dominance and chasing. Lastly, SNA metrics did not correlate across days, suggesting fluidity in social dynamics over time. Ongoing analysis of brain tissue will reveal steroid neuroanatomical profiles that will be related to SNA metrics to determine how aspects of social structure may be reflected in brain phenotype.

Disclosures: C.J. Esquilin-Rodriguez: None. **C. Lee:** None. **A.M. Kelly:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.14/X3

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 5R03 HD101863

Title: Arginine vasotocin receptor density is differentially regulated across telencephalic regions during behavioral-sex transformation in the sex-changing fish, Thalassoma bifasciatum

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Abstract: Bluehead wrasses are an excellent model for investigating the neuronal plasticity mechanisms that underline transformations of behavioral sex phenotype. Wrasses on small patch reefs consist of many initial-phase females (IP) and one terminal-phase male (TP). If the TP male is removed, the most dominant IP female will transform into a new TP male. Transformation of behavioral sex can occur within 1-2 days of removing the TP male, whereas at least two weeks are required for full gonadal and color transformation. The “priming/gating” hypothesis postulates that IP females become more competent to show male-typical courtship behaviors as they ascend the social dominance hierarchy; the presence of a TP male appears to inhibit this expression. A primary objective of our research is to elucidate the neural bases of male-typical courtship behavior and its regulation. Glutamate iontophoresis experiments have identified several forebrain regions that elicit coloration changes and motor behaviors that are characteristic of male-typical courtship behavior. For example, stimulation of the supracommissural nucleus of the ventral telencephalon (Vs) elicits coloration changes similar to “opalescence” seen during male-typical courtship. On the regulatory side, arginine vasotocin receptors (AVT) in preoptic areas are upregulated in TP males and appear to promote competency to display male-typical

courtship behavior. In our current study, we used RNAscope *in situ* hybridization to further examine how expression of genes for AVT receptors changes across forebrain regions as IP females achieve competency for displaying male-typical courtship behavior. For non-dominant IP females, expression of the gene for V1a2-type AVT receptors was high in the central nucleus of the ventral telencephalon (Vc), but low in most other forebrain regions. In contrast, in dominant IP females that were competent for displaying male-typical courtship behavior and TP fish, V1a2 expression was strongly downregulated in Vc and upregulated in rostral Vs, a medial area of the dorsal telencephalon (Dm2) and magnocellular medial preoptic nucleus (PMm). Transformation in behavioral-sex phenotype, therefore, is accompanied by a down-regulation of V1a2 receptors in Vc and upregulation in Vs, Dm2, and PMm regions. We, therefore, propose that Vc may act to inhibit activity in courtship-related forebrain regions, and thereby suppress expression of male-typical courtship behavior in unprimed fish; downregulation of AVT receptors in Vc may release these regions from inhibition and thereby promote behavioral sex transformation.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.15/X4

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NSERC PGSD
NSF Grant 1933166

Title: Plasticity in neuropeptide signaling: Preoptic area galanin immunoreactivity differs by reproductive state in a seasonally breeding teleost fish

Authors: *S. L. GRAY[1], K. KOBI[2], P. M. FORLANO[2], J. A. SISNEROS[1];
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Abstract: Galanin is an evolutionarily conserved neuropeptide hormone that regulates feeding and reproductive behaviors across vertebrates. Preoptic area (POA) galanin is implicated in modulating parental care in both rodents and teleost fish. The plainfin midshipman (*Porichthys notatus*), a seasonally breeding teleost, provides paternal-only care and is a valuable model species for investigating the neuroendocrine underpinnings of reproductive behaviors. Galanin-expressing neurons have been shown to exhibit sex-specific expression patterns, with increased galanin activity in courting, but not caregiving, male midshipman. However, seasonal plasticity of the POA galanin system remains poorly understood. In the current study, we examined galanin immunoreactivity in the POA of reproductive and non-reproductive adult male midshipman fish. Our results reveal significant seasonal differences in galanin expression

patterns across several subdivisions of the POA. Specifically, we observe an increase in the number of POA galanin-expressing neurons in reproductive males compared to non-reproductive males. These findings advance our understanding of the neuropeptidergic mechanisms underlying complex reproductive social behaviors in an ecologically relevant teleost fish model. Furthermore, they illustrate the dynamic plasticity of the vertebrate neuroendocrine system, which supports adaptive behavioral responses to seasonal reproductive demands.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.16/X5

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: JSPS Grant 22H00396

Title: Analysis of male-type sexual behavior in progranulin gene modified rats

Authors: *T. MATSUWAKI, A. TAKABATAKE, K. YAMANOUCHI;
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Abstract: Progranulin (PGRN) is a multifunctional growth factor that is widely expressed throughout the body. We previously identified PGRN as one of the genes that plays important roles in brain sexual differentiation and the regulation of male sexual behavior in rodents. To investigate the role of PGRN in these processes, we generated two strains of PGRN mutant rats using CRISPR/Cas genome editing system with improved-Genome editing via Oviductal Nucleic Acids Delivery (iGONAD) method: the Δ34 line with out-of-frame deletion and the Δ126 line with in-frame deletion, both in exon 2. As a result of immunohistochemical analysis, the Δ34 (-/-) rats showed a complete loss of PGRN expression in the brain. In contrast, Δ126 (-/-) rats had a comparative expression level of PGRN to that of wild-type (WT) rats. Serum PGRN concentration was undetectable in Δ34 (-/-) rats. In Δ126 (-/-) rats, it was detectable but significantly lower than that in WT rats. Male rats of both lines were mated with estrogen-tube-primed estrus female rats for 30 min, and their male-type sexual behaviors were evaluated. There were no significant differences between Δ34 (-/-) and WT rats. However, Δ126 (-/-) rats exhibited decreased frequency of mount, intromission, and ejaculation compared to WT rats. The size of the sexually dimorphic nucleus in the preoptic area (SDN-POA), a brain region involved in regulating male sexual behavior, was not significantly different between the Δ126 strain and wild-type rats. Blood testosterone levels were unaffected in both strains. These findings suggest that PGRN contributes to the regulation of male sexual behaviors through a pathway that is independent of testosterone secretion and SDN-POA size. The suppression of male sexual behavior in the Δ126 strain, but not in the Δ34 strain, indicates the existence of an alternative mechanism that compensates for PGRN function in its absence.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.17/X6

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Motivated behaviors: effects of the interaction between sexual behavior and voluntary excercise in female rats

Authors: *A. MENDEZ GUERRERO[1], R. G. PAREDES[2,1];
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Abstract: Paced mating and voluntary wheel running are examples of motivated behaviors in rodents, modulated by neural circuits involved in motivation and reward. Both behaviors induce plastic changes in the brain at both structural and functional levels. This study aimed to evaluate the effects of performing these two behaviors jointly for several weeks and after a period of behavioral suppression. Ovariectomized female rats supplemented with estradiol and progesterone were assigned to the following experimental groups: (1) voluntary wheel running (WR) (1 h/day, 5 days/week); (2) paced mating (PM) (1 h/week); and (3) dual-behavior group (PM/WR), with access to voluntary wheel running (1 h/day, 5 days/week) and paced mating in the same session (1 h/week). The protocol was maintained for 10 weeks, followed by a 4-week period of behavioral cessation and one final behavioral test in week 15. Results showed that the WR group exhibited greater running distances compared to the PM/WR group, suggesting that sexual behavior may modulate voluntary activity over time. Following the behavioral suspension phase, a reduction in physical activity was observed during week 15. In contrast, no significant differences were found in sexual behavior expression between the PM and PM/WR groups, indicating that the addition of exercise does not alter the sexual behavior pattern when both behaviors are performed within the same session. Furthermore, sexual behavior remained unchanged after the interruption of the behavioral tests. The data show that sexual behavior is stable across conditions involving exercise and interruption of behavioral testing, while the simultaneous performance of paced mating influences voluntary wheel running. The results indicate that females can display two motivated behaviors in the same session.

Disclosures: A. Mendez Guerrero: None. **R.G. Paredes:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

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INPER 2022-1-13 (NF.D)

Title: Socio-sexual behaviors promote spinogenesis in the nucleus accumbens of adult female prairie voles

Authors: A. E. CASTRO[1], D. AVILA[2], F. CAMACHO[4], R. G. PAREDES[5], N. F. DIAZ[3], *W. PORTILLO[6];
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Abstract: Prairie voles (*Microtus ochrogaster*) are monogamous rodents that establish life-long pair-bonds. Since social and sexual experiences modulate brain plasticity, the present study aimed to elucidate in female voles if social exposure to a male or social cohabitation with mating, which leads to pair-bonding, modulates spinogenesis in the medium spiny neurons (MSNs) in the nucleus accumbens (NAc). Females were randomly assigned to one of the following groups: 1) Control (C), voles that cohabited with a familiar female in a clean cage; 2) Social exposure (SE), females housed in a cage divided into two equal compartments by an acrylic screen with small holes. The experimental vole was placed in one of the compartments, and a male in the opposite one. Therefore, females were exposed to sensory cues from an adult male without mating, and 3) Social cohabitation with mating (SCM) females were allowed to mate to induce pair-bonds. The NAc core and shell were processed for Golgi-Cox staining. Our results showed that MSN from SE and SCM groups had higher spine density than C females and a differential density of spine subtypes in the core and shell. Furthermore, only the SE condition induced an increment in MSN dendritic length and arborization in the core and shell regions. These findings demonstrate that male exposure and mating that promote pair-bonding modulate spinogenesis in the NAc.We thank Nydia Hernández, Ericka De los Ríos, Lorena Gaytán, Santiago Figueroa, Deisy Gasca, Carlos Lozano, Martín García, and Alejandra Castilla for their excellent technical assistance.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: PSTR398.19/Web Only

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: Conahcyt CF-2023-G-289

Title: Lordosis behavior elicited by kisspeptin is mediated by several protein kinases

Authors: *R. DOMÍNGUEZ ORDÓÑEZ[1], J. L. ENCARNACIÓN SÁNCHEZ[2], M. GARCIA-JUÁREZ[3], C. AGUILAR PEREZ[4], O. GONZÁLEZ FLORES[5];
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Abstract: Intact female rats show lordosis behavior at the afternoon-night of proestrus, just after have reached a peak of estradiol and progesterone. However, the neural circuits that allows this response shows a fine-tune regulation, where other compounds interact by different intracellular pathways. One of these compounds is kisspeptin, a peptide hormone that releases GnRH (a well-known lordogenic compound). The present work explores the protein kinases Src, MAPK, PKA and PKC role in the facilitation of lordosis behavior, because in other models has been showed that kisspeptin can elicit GnRH release. For this purpose, we design two experiments. In the first one, we explore the effect of PD98059 (MAPK inhibitor), and PP2 (Src inhibitor). In the second experiment, we explore the effect of Rp-cAMPs (PKA inhibitor) and bisindolylmaleimide (PKC inhibitor). Therefore, 53 Sprague Dawley female rats, of 200 g, where *OVX* and *i.c.v.* implanted with a stainless steel cannula of 17 x 22g following the stereotaxic coordinates of the Atlas of Paxinos and Watson (2006). For experiment 1, 26 rats received an initial subcutaneous dose of 5µg of EB, followed at thirty-five h later by the *i.c.v.* administration of PD98059 (n=10), PP2 (n=8) or its vehicle, DMSO (n=8). Thirty min later, all of these rats received kisspeptin. In experiment 2, we use 27 female rats, where at 0 h received 5 µg of EB, followed at 39.5 h by the *i.c.v* injection of Rp-cAMPs (n=9) or bisindolylmaleimide (n=8) or its vehicle, saline (n=10). At thirty min later, all females received kisspeptin. These results suggest that all of these intracellular pathways are involved in lordosis behavior elicited by kisspeptin, because the *i.c.v.* injection of the different protein kinase inhibitors diminished lordosis behavior at the different times tested. Thus, the proposed mechanism by which kisspeptin is eliciting lordosis could be through GnRH releasing.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: G.02. Neuroendrocrine Processes and Behavior

Support: Technical support: Camacho F. and González Gallardo A.
DGAPA/PAPIIT grant IA208624

Title: Expression of KISS1 in different brain areas of male and female rats depending on sexual reward

Authors: *M. BEDOS[1], I. RUFINO[2];
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Abstract: Kisspeptin is a potent regulator of the hypothalamus-pituitary-gonads axis and studies have shown that the peptide increases sexual receptivity in female mice and increases the number of ex-copula erections in male rats. Recently, we demonstrated that kisspeptin induces a positive affective (PA) state and that its antagonist blocks the PA state elicited by paced mating in females. The aim of the work was to determine whether sexual behavior stimulates kisspeptin expression in brain areas related to sexual behavior and reward. Four groups of adult Wistar rats of both sexes were used: Control: animals were placed in the mating box for 1h; Paced mating: animals copulated for 1h, with the possibility of regulating the interaction with the opposite sex; Non-Paced mating: animals copulated for 1h, without the possibility of regulating the interaction with the opposite sex; Sexually relevant olfactory stimulus: animals were exposed in the mating box to sawdust of the opposite sex for 1h. All females were previously ovariectomized and induced to estrous by s.c. injections of estradiol benzoate and progesterone 48h and 4h before the tests, respectively. Parameters of sexual behavior were recorded. The subjects were sacrificed by decapitation at the end of the behavioral tests. The anterior ventral periventricular area (AVPV), arcuate nucleus (ARC), amygdala (AMG) and olfactory bulbs (BO) were dissected. After RNA isolation and cDNA synthesis, expression of the KISS1 mRNA was determined by qPCR in duplicates. Preliminary results show that mating did not change KISS1 expression in either the ARC or the AVPV.

Disclosures: M. Bedos: None. **I. Rufino:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.21/X9

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 5K08AG070377-03

Title: Differential effects of Oxtr signaling across tissues and ages in the socially monogamous prairie vole

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Abstract: Social relationships are increasingly recognized to have a profound influence on human health. Social isolation and loss of social attachments have been associated with increased risk of numerous adverse health outcomes, including cardiovascular disease, diabetes, and all-cause mortality. Neuroendocrine signaling, particularly oxytocin signaling, is implicated in regulating organ and tissue function throughout the lifespan and is a known mediator of attachment behavior across species. However, a mechanistic understanding of how oxytocin regulates the links between social attachment behavior and peripheral physiological health is unclear, and has been limited by the lack of genetic animal models displaying long-term adult social bonds. The prairie vole is a unique rodent species that exhibits life-long pair bonds, making it an ideal model system by which to study attachment. Using CRISPR/Cas9-based molecular genetic tools in the prairie vole, we have generated mutations in genes of interest, including the oxytocin receptor (*Oxtr*). Using this system, we found that loss of Oxtr signaling delays formation of adult pair bonds and leads to distinct changes in transcriptional signatures in brain regions implicated in attachment and other social behaviors. Using RNA-Seq from brain samples from WT and *Oxtr-/-* voles, we identified baseline differences in gene expression in the nucleus accumbens due to loss of *Oxtr*. Pathway analysis implicates known oxytocin receptor signaling genes, as well as genes related to other GPCR and non-neuronal signaling pathways. Further, oxytocin receptors are expressed throughout the body, in multiple organ systems, regulating cardiovascular and metabolic function, fluid homeostasis, and food intake. Our overarching hypothesis is that oxytocin signaling is required for the broad physiological benefits associated with social attachment and regulates peripheral physiological changes with pair bonding across the lifespan. Functional changes with social attachment or social stressors may reflect mechanisms of resilience or vulnerability respectively in physiological systems regulated by oxytocin. Thus, we aim to clarify the role of oxytocin signaling in coordinating function across physiological systems and social states throughout the lifespan.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: PSTR398.22/X10

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: MH121829

Title: Effects of Social Stress on Oxytocin Neuron Gene Transcription in the Hypothalamic Paraventricular Nucleus of California Mice

Authors: *A. N. CHRISMAN[1], S. ARCHDEACON[2], A. SERNA GODOY[3], B. C. TRAINOR[4]; [1]Univ. Of California Davis Neurosci. Grad. Program, Davis, CA; [2]Univ. of California, Davis, Davis, CA; [3]UC Davis, Davis, CA; [4]Univ. of California -Davis, Davis, CA

Abstract: Psychosocial stress is a risk factor for psychiatric disorders such as depression and anxiety. Understanding how psychosocial stress alters brain and behavior will help develop targeted interventions to mitigate its detrimental effects, improving mental health outcomes and alleviating societal burden. Social defeat stress (SDS), in which an animal loses an aggressive interaction, models the impact of social stress on behavior. The stress paradigm in California mice (*Peromyscus californicus*) elicits behavioral responses to stress that are also seen in humans, including reduced social approach and increased vigilance. These responses are more persistent in females, are translationally relevant behaviors because they are found in disorders such as social anxiety disorder. During episodes of SDS, oxytocin neurons in the paraventricular nucleus of the hypothalamus (PVN) are activated in both male and female *P. californicus*. Notably, stressed females exhibit increased activation of anterior PVN oxytocin neurons compared to controls during a social interaction test two weeks post-stress. This heightened activity is negatively correlated with social interaction, suggesting that these neurons contribute to social avoidance following stress exposure. These findings support the idea that stress-induced plasticity in anterior PVN oxytocin neurons may encode social vigilance and avoidance, providing a neurobiological basis for the context-dependent effects of oxytocin on social behavior. Single-nucleus RNA sequencing (snRNA-seq) of the *P. californicus* PVN was performed using the 10x Genomics Nuclei Isolation kit (N= 8 stressed females, 8 control females, 4 stressed males, and 4 control males, yielding 87,250 nuclei) to search for upregulated transcripts in PVN oxytocin neurons. Biologically relevant, plasticity-related transcripts were identified to be upregulated by SDS in female oxytocin neurons using pseudobulk and limma-voom differential expression analysis in the Seurat package. Specifically, 30 transcripts under the gene ontology cellular component term “presynapse” (FDR=7.25E-20) and 31 transcripts under the term “postsynapse” (FDR=8.48E-19) were implicated. Ongoing in-situ hybridization validation and tract tracing analysis will shed light on functional relevance of these upregulated transcripts. Identifying the molecular changes occurring in stress-sensitive brain areas may reveal how psychosocial stress shapes future behavior, inspiring novel therapeutic targets.

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Poster

PSTR398: Parental and Socio-Sexual Behaviors

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.23/X11

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: The Good Nature Institute

Title: The role of oxytocin receptor in neural activity during forced social interactions

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Abstract: Whether a mole crawling through an underground tunnel or a person walking down a narrow office hallway, brief head-on engagements in small spaces where one participant dominates are a part of life for many species. Oxytocin and its receptor (OXTR) mediate social behavior such as maternal bonding or social recognition, but their role in social dominance is relatively unexplored. The tube test models forced social interactions in rodents, wherein animals are placed on opposing sides of a tube and proceed toward one another - the tube is too small for them to turn around, so they must either back out or force their opponent out. Thus, the subordinate loser exits rear-first while the dominant winner exits head-first. In this study, adult mice on a C57BL/6J background were trained to proceed through an empty tube ten times per day for two days. On day three after a habituation period mice proceeded once through an empty tube (alone) or against a pre-determined opponent (dyad); a dyad was made of two familiar mice (shared home cage) expressing the same OXTR genotype (two [WT], one [HET], or zero [KO] copies of OXTR). Following a 90-minute isolation, mice were euthanized with Euthasol (50 mg/kg), perfused with 4% paraformaldehyde, and harvested. In this study we used c-Fos immunohistochemistry to quantify differences in contextual neuronal activity. We investigated c-Fos presence in the paraventricular nucleus (PVN) of the hypothalamus because it is an oxytocin-producing region and potential site of upstream cascade effects by the ligand. FIJI (ImageJ) was used to measure cell counts, nuclear area, PVN size, and cells per area. Our study followed a 3x2x2 design using OXTR genotype (WT, HET, KO), sex (males [n=34] and females [n=38]), and exposure (alone or dyad) as predictor variables (n=4-10 per group), without regard to winner or loser status. We hypothesized that genotype would play a significant role in neuronal activation with greater c-Fos counts in dyads but instead found that females have higher PVN activity than males with no genotype or exposure interactions. Additionally, using the freehand selected ROI, we found that WTs have a larger PVN than KO but not HET mice.

Disclosures: R. Ochoa: None. **E.A.D. Hammock:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.24/X12

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: JSPS KAKENHI 23KJ0281
JSPS KAKENHI 22H01099

Title: Role of estrogen receptor beta in the medial preoptic area in female reproductive responses

Authors: *L. KOGURE[1,2], T. MURAKAWA[1], S. OGAWA[1], M. NAKATA[1];
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Abstract: Estrogen receptor beta (ERβ) is abundantly distributed in neural circuits for female reproductive function. Among these, the medial preoptic area (MPOA) plays a central role in coordinating both behavioral and hormonal responses during mating. However, the specific role of ERβ in the MPOA in female reproduction is not fully understood. In the present study, we knocked down ERβ in the MPOA to investigate its role in regulating female sexual behavior and the induction of pseudopregnancy. Pseudopregnancy is a progesterone-dominant state triggered by non-conceptive vaginal stimulation from an infertile male.Gonadally-intact female C57BL/6N mice were bilaterally injected with AAV vectors encoding either ERβ-targeting shRNA (βKD, n = 10) or control luciferase shRNA (control, n = 10), with injection sites aimed at the MPOA. Starting at two weeks after injections, females were confirmed their estrous cycles with vaginal smears. On the day of proestrus, the females were then tested with a sexually experienced vasectomized male in his home cage until receiving 16 intromissions or reaching 30 min. Lordosis behavior was scored for each male mount as either present or absent, and the lordosis quotient (LQ) was calculated as the number of lordosis responses divided by the number of mounts, multiplied by 100. Vaginal smears were collected daily after the sexual behavior test to examine the induction of pseudopregnancy. Pseudopregnancy was determined by the presence of leukocytes in vaginal smears for at least four consecutive days within the following 12 days.The results showed that ERβ knockdown group showed significantly higher LQ and increased pseudopregnancy incidence compared to the control group. Notably, histological analysis revealed viral spread not limited to the MPOA but extending to areas such as the lateral septum and bed nucleus of the stria terminalis. These findings suggest that ERβ signaling in the MPOA and adjacent forebrain regions modulates female sexual receptivity and suppresses the transition to pseudopregnancy.

Disclosures: L. Kogure: None. **T. Murakawa:** None. **S. Ogawa:** None. **M. Nakata:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.25/X13

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Gonadal steroid-independent male sexual behavior in B6D2F1 male mice is associated with decreased anxiety-like behavior

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Abstract: In many mammalian species, gonadal steroids, particularly testosterone, play a critical role in modulating numerous social behaviors, including both reproductive activity and emotional regulation. Testosterone is both necessary and sufficient to activate male sexual behavior and has well-documented anxiolytic effects. Yet, some individuals exhibit sustained sexual behavior and emotional resilience in the absence of circulating androgens, suggesting the presence of gonadal steroid-independent mechanisms. However, the pathways that maintain motivated behavior and affective stability in hypogonadal conditions remain poorly understood. The B6D2F1 hybrid mouse provides an effective model to investigate these mechanisms, as one-third of these males demonstrate sexual behavior long after castration (herein referred to as "maters"). Preliminary results from our lab revealed that maters, relative to non-maters, displayed significantly reduced anxiety-like behaviors in both the Open Field Test and Elevated Plus Maze. Thus, the presence of male sexual behavior coupled with reduced anxiety-like behaviors in the maters may suggest similar underlying steroid-independent mechanisms at play. To validate our pilot data, we will conduct a full-scale study incorporating the previous behavioral assays alongside measuring serum corticosterone concentrations and gene expression of biological markers associated with anxiety modulation. This research leverages an innovative model to dissect gonadal steroid-independent mechanisms underpinning resilience, offering insights that may inform our understanding of hypogonadal states, affective disorders, and novel avenues of anxiety regulation.

Disclosures: J. Park: None. **A. Wiles:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR398.26/X14

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: CONAHCYT CF-2023-I-368
CONAHCYT 843707

Title: Paradoxical long-term effects of perinatal paracetamol exposure on adult sexual partner preference, anxiety-like behavior, and steroid hormone levels

Authors: A. D. GUZMÁN MONTEMAYOR[1], J. SIERRA[1], M. BARRADAS-MOCTEZUMA[1], A. A. CORONA-MORALES[2], J. MANZO[1], R. TOLEDO-CARDENAS[1], M. HERNANDEZ[1], D. HERRERA-COVARRUBIAS[1], ***G. A. CORIA-AVILA**[3];
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Abstract: Paracetamol is used during pregnancy due to its apparent safety at therapeutic doses. Its mechanism of action involves the inhibition of COX-2 and COX-3 enzymes, which play roles in the modulation of pain and inflammation, as well as in brain masculinization during the

perinatal period in males.Recent studies suggest that administration during critical developmental windows may act as an endocrine and behavioral disruptor, reducing partner preference for females. However, other studies have paradoxically reported an increase in this preference and a higher frequency of consummatory behaviors. Therefore, conclusive clinical evidence regarding its long-term effects and the mechanisms underlying these opposing outcomes is still lacking.In this study, we investigated the effects of paracetamol as a potential neuroendocrine disruptor of the male sexual brain when administered during critical stages of prenatal and neonatal development. For the prenatal group, pregnant Wistar rats received 60 mg/kg of paracetamol or saline every 12 hours from gestational day 16 to 20. In the neonatal group, male pups received the same dose from postnatal day 1 to 5. All animals were weaned on day 21 and housed in groups of 4-5 until adulthood.On postnatal day 70, half of the males in each group were exposed to receptive females to acquire sexual experience, while the other half remained sexually inexperienced. On days 94 and 95, anxiety- and depression-like behaviors were assessed using the elevated plus maze and the forced swim test, respectively. On day 98, sexual partner preference was evaluated, and on day 107, blood was collected via cardiac puncture to determine serum testosterone and corticosterone levels using the ELISA technique.The results showed that both prenatal and neonatal exposure to paracetamol facilitated female-oriented partner preference in adulthood, increasing physical contact time, as well as mounting and intromission frequency, while reducing ejaculation latency. Interestingly, only males prenatally exposed to paracetamol exhibited a lower anxiety index, higher serum testosterone levels, and reduced corticosterone levels. We discuss these paradoxical effects within the broader context of neuroendocrine alterations induced by perinatal paracetamol exposure.

Disclosures: A.D. Guzmán Montemayor: None. **J. Sierra:** None. **M. Barradas-Moctezuma:** None. **A.A. Corona-Morales:** None. **J. Manzo:** None. **R. Toledo-Cardenas:** None. **M. Hernandez:** None. **D. Herrera-Covarrubias:** None. **G.A. Coria-Avila:** None.

Poster

PSTR398: Parental and Socio-Sexual Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: PSTR398.27/X15

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 1R01HD114146-01A1

Title: Experience-dependent Trpc5 Expression in MPOA Esr1 Neurons Governs Parental Behavior in Male mice

Authors: *Y. LI[1], Q. LIU[1], S. FAROOQI[2], Y. XU[1];
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Abstract: Parental interactions are essential for offspring survival. In species where males contribute to parenting, paternal behavior enhances offspring fitness by providing necessary resources. Male mice switch from pup-directed aggression as virgins to caregiving after becoming fathers. However, the underlying cellular and molecular mechanisms remain largely unknown. In this study, we found that virgin male mice either attacked or ignored pups (50% attacked; 50% ignored), whereas males retrieved pups (100% retrieved) after becoming fathers. Whole-brain mapping revealed a significant increase in the expression of Transient Receptor Potential Channel 5 (Trpc5), a calcium ion channel, in medial preoptic area (MPOA) estrogen receptor α (Esr1)-expressing neurons of fathers (49.2 ± 5.5%), compared to virgin males (11 ± 0.85%). We have shown that loss-of-function mutation in Trpc5 impairs pups' retrieval in dams. Here, we found that approximately 50% of Trpc5 mutant fathers failed to retrieve pups. Also, deletion of Trpc5 in MPOA Esr1 neurons led to a pup retrieval deficit in 53% of fathers, while 100% of control fathers retrieved pups. Conversely, Trpc5 overexpression in MPOA Esr1 neurons induced pup retrieval in virgin males (64% retrieved), compared to control virgin males (45% attacked; 55% ignored). We also found that chemogenetic activation of MPOA Esr1 neurons reduced pup-directed attack rates from 57% to 0% in virgin male mice. To determine whether MPOA Esr1 neuron activity is altered during this process, we performed *in vitro* electrophysiological recordings. We found that MPOA Esr1 neurons in fathers exhibited higher firing frequency (5.70 ± 0.48 s^{-1}) and were more excitable after current injections than virgin males (2.82 ± 0.69 s^{-1}). Deletion of Trpc5 in MPOA Esr1 neurons reduced firing frequency in fathers (0.52 ± 0.40 s^{-1}) compared to control fathers (5.02 ± 0.77 s^{-1}), whereas Trpc5 overexpression increased firing frequency in virgin males (7.65 ± 1.10 s^{-1}) relative to control virgin males (2.59 ± 0.54 s^{-1}). *In vivo* fiber photometry recordings revealed that MPOA Esr1 neurons exhibited a stronger response to pups in behaving fathers compared to virgin males. Consistently, Trpc5 deletion attenuated pup-evoked activity of MPOA Esr1 neurons in fathers, whereas Trpc5 overexpression in virgin males increased their responsiveness to pups. Together, these findings identify Trpc5 as a key molecular and MPOA Esr1 neurons as the cellular substrate for controlling paternal behavior. Our study provides new insights into the neurobiological mechanisms underlying male caregiving and establishes a critical role for Trpc5 in regulating paternal behavior.

Disclosures: Y. Li: None. **Q. Liu:** None. **S. Farooqi:** None. **Y. Xu:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: G.02. Neuroendrocrine Processes and Behavior

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Eunice Kennedy Shriver National Institute Of Child Health & Human

Development of the National Institutes of Health under Award Number F32HD111327

Title: Plasma oxytocin levels in the Russian farm foxes

Authors: *O. T. REILLY[1], A. V. KUKEKOVA[2], A. V. KHARLAMOVA[3], R. GULEVICH[4], D. SHEPELEVA[5,3], A. V. VLADIMIROVA[6], L. N. TRUT[3], E. E. HECHT[1];
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Abstract: The farm-fox study at the Institute for Cytology and Genetics at Novosibirsk has bred generations of foxes (*Vulpes vulpes*) for the past several decades based on behavioral responses when approached by an unfamiliar human. This has resulted in three different strains of foxes that exhibit separate social behavioral phenotypes: one that shows “tame” social approach behaviors, one that shows aggression or avoidance behaviors, and a control strain that has been bred without selection on behavior. Oxytocin is a neuropeptide hormone that plays a critical role in social attachment and affiliative behaviors. There is evidence that oxytocin inhibits hypothalamic-pituitary-adrenal (HPA) axis activity, resulting in anxiolytic effects during bouts of social stress. It has been hypothesized that oxytocin may be implicated in the biological mechanisms of domestication. The Russian farm-foxes serve as an ideal model to investigate the role of oxytocin in the domestication process. Here we investigate baseline levels of plasma oxytocin in the three groups of Russian farm-foxes. We analyzed 42 fox plasma oxytocin samples (*tame: n=18; aggressive: n=12; control: n=12*) using a commercially available enzyme immunoassay kit. There were no significant differences in baseline plasma oxytocin level between the tame, aggressive, and control groups of foxes. This suggests that behavior differences in reaction to human interactors might be due to oxytocin release during social interactions, and/or to changes in other brain systems. Future work is necessary to determine whether such differences are observed.

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Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.02/Y1

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH NIGMS R35 GM119844
Local Initiative For Excellence (L.I.F.E.) Foundation Research Grant
NIH NINDS R01 NS120984

Title: Untangling the complexity of a versatile neuromodulator: genetic and functional dissection of Drosophila Octopaminergic neurons

Authors: *V. FAJNER[1], L. PALACIOS CASTILLO[2], E. YALBIR[1], J. LEE[1], V. MORAD[1], K. ISHII[1], M. A. FRYE[2], K. ASAHINA[1,3];
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Abstract: Aggression is an ethologically relevant social behavior observed across species. Deciphering the mechanisms that regulates aggression is essential for understanding how the nervous system modulates this behavior to maximize fitness, and how failures in this regulation may contribute to psychiatric conditions and neurodevelopmental disorders.Various neuromodulators have been implicated in the regulation of this complex social behavior. Among them, the biogenic amine octopamine (OA) - the invertebrate analogue of mammalian norepinephrine - is particularly intriguing, as it may operate at multiple levels of aggression control. OA neurons in *Drosophila melanogaster* have been shown to both promote [Hoyer et al., 2008; Zhou et al, 2008] and suppress [Ishii et al., 2022] aggression. Furthermore, OA has been implicated in the regulation of diverse behaviors, including courtship [Huang et al., 2016], feeding [Youn et al., 2018], persistent odor tracking [Sayin et al., 2019], and reward processing [Burke et al., 2012; Hermanns et al., 2022].*Drosophila* OA neurons comprise at least 27 anatomically distinct subtypes [Busch et al. 2009], suggesting that specific subtypes may control discrete behaviors. Although results of previous research (ex. Zhou et al., 2008; Burke et al., 2012; Sayin et al., 2019; Hermanns et al., 2022) are consistent with this hypothesis, subtype-specific effects on a given behavior has been challenging to investigate because of the lack of a method to systematically manipulate each OA neuronal subtype. In this study, we leveraged the split-GAL4 driver system [Luan et al., 2006] to gain genetic access to individual OA subtypes. We have isolated a set of split GAL4 lines that collectively label 24 out of the 27 known OA subtypes. Using the multicolor Flip-Out technique [Nern, et al., 2015], we resolved these subtypes at the single-cell level and assessed their roles in modulating aggression intensity in socially experienced and socially naïve flies.By disentangling the diversity and function of octopaminergic subtypes, this work contributes to a mechanistic understanding of how specific neuromodulators sculpt complex social behaviors. Furthermore, given the evolutionary conservation of biogenic amines, this study may offer new insight into the cellular and molecular architecture of aggression regulation in other invertebrates, with potential relevance for understanding the neural basis of noradrenergic neuromodulation in vertebrates.

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Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.03/Y2

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Characterizing the Role of a Y-linked Histone Demethylase, Kdm5d, In Sex Biased Behaviors

Authors: *M. MIYASATO[1], N. C. TRONSON[2], S. IWASE[3];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Dept. of Psychology, Univ. of Mchigan, Ann Arbor, MI; [3]Human Genet., Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract: Sexually biased behaviors, such as aggression in males and maternal behavior in females, arise during development. *Sry*, a gene on the Y-chromosome, is thought to be the main driver of these sex biases by initiating the development of the testis. A surge of testosterone is released from the newly developed testis in utero; testosterone is aromatized into estrogen and in turn creates the "male patterned" brain. Though *Sry*-mediated brain masculinization is well understood, the other genes on the Y-Chromosome have yet to be implicated in the context of brain development. Y-linked genes can be separated into two classes: testis-specific genes and X-Y linked homologs that are expressed ubiquitously in the body. A candidate X-Y linked gene pair, *Kdm5c* and *Kdm5d,* both encodes for histone H3 lysine 4 di- and tri- demethylases expressed in the brain. *Kdm5c,* the X-linked homolog, has previously been demonstrated to be critical for brain development. *Kdm5c* Knockout (KO) male mouse models show heightened aggression and learning impairments, however the role of its Y-linked homolog, *Kdm5d*, has not been unveiled. Towards this question, we created a *Kdm5d* null mouse model using CRISPR and characterized their behavior. We hypothesize that Kdm5d mediates male-specific aggressive behaviors, and knocking out *Kdm5d* will result in a decrease in aggression and social dominance. Preliminary data show a decrease in aggression using the resident intruder paradigm compared to its X-linked counterpart *Kdm5c* KO animals. We further found the *Kdm5d* KO male mice were less dominant than Wild Type male mice when testing social dominance, suggesting a difference in function and gene regulation of these X-Y linked homologs. Ongoing studies aim to analyze what exact genes *Kdm5d* are responsible for regulating in the brain and how this X-Y linked homolog contributes to sex biased behaviors.

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Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.04/Y3

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Maternal defense against intruders changes subsequent maternal behavior and alters neural circuitry

Authors: P. A. ROBINSON-DRUMMER[1], S. M. LUZ[2], G. A. BARR[2], ***S. BHATNAGAR**[3]; [1]Psychology, Haverford Col., Haverford, PA; [2]CHOP, Philadelphia, PA; [3]Children's Hosp. of Philadelphia and Univ. of Pennsylvania, Philadelphia, PA

Abstract: During the postnatal period, maternal behavior adapts to the changing needs of the pups with high levels of maternal nurturing and vigilance. Maternal behaviors shift as environmental and hormonal influences alter activity in neural circuits that switch between nurturing in the presence of pups and defensive behaviors in the presence of threats. Although much is known about the neurobiology of nurturing maternal behaviors, less is known about how the stress of defending the nest and litter changes the dam-pup relationship and the dam's long-term neurobiology. The current experiment examined how exposure to an intruder into the dam's cage alters the pup-directed and non-pup-directed behavior of the dam following defeat of the intruder. Once pups were weaned, we also measured dam hormonal and neural responses to acute restraint stress. Several groups of lactating females were tested. Resident (RES) dam maternal behavior was measured after 1 day or 5 days of defeat of a female intruder with pups temporarily removed for 1hr during the exposure to the female intruders. The intruder was typically defeated by the lactating female. Control (CON) lactating females had their pups removed for 1 hour without exposure to an intruder in their cage. Naïve groups were also included in which no manipulations were conducted. Following one day of defeat of an intruder, rough handling and transport of pups was increased in RES compared to CON whereas nursing and grooming patterns were largely unchanged. After 5 days of exposure to a different intruder every day, rough behaviors toward the pup remained elevated in RES dams relative to CON. Additionally, levels of rearing increased in RES dams suggesting hypervigilance. These behavioral changes were most pronounced during the first five minutes after direct exposure to the intruder ended and pups were returned to the dam. Ninety minutes after acute 30min restraint on day 6, neuronal activity in the medial prefrontal cortex and basolateral amygdala, as assessed by numbers of Fos-expressing cells, was increased in both RES and CON relative to naive females. However, activity in dorsomedial and dorsolateral periaqueductal gray (PAG) was increased in RES relative to all other groups suggesting these structures are important for defensive aggression uniquely engaged by defending against an intruder. After weaning, RES dams showed reduced CORT responses to restraint relative to CON suggesting long-term alterations in stress regulation. These results suggest both immediate and long-term shifts in dam neurobiology induced by defensive behaviors that impact the quality of maternal care and dam emotional regulation.

Disclosures: P.A. Robinson-Drummer: None. **S.M. Luz:** None. **G.A. Barr:** None. **S. Bhatnagar:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

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Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIGMS Grant R35GM142799
Beckman Young Investigator Award
University of Houston National Research University Fund Startup
R0503962

Title: Investigating the role of somatostatin in regulating social dominance in a cichlid

Authors: *L. A. SAENZ, S. YOUNG;
Univ. of California Los Angeles, Los Angeles, CA

Abstract: Abstract: Somatostatin (SST) is known for its role in metabolism, growth and feeding. However, there is evidence SST modulates social behaviors such as aggression. In *Astatotilapia burtoni*, SST appears to influence aggressive behaviors associated with social status; however, its specific behavioral functions are unknown. Using single-cell RNA sequencing (scRNA-seq) of the hypothalamus, we revealed that dominant (DOM) males expression more *sst1.1* in *grin2ab+* glutamatergic neurons compared to subordinate (SUB) males. We performed hybridization chain reaction (HCR) to validate these findings and successfully detected *sst1.1* expression within *grin2ab+* cells in the hypothalamus. Ongoing analysis is focused on quantifying *sst1.1* expression within *grin2ab* positive cells in both DOM and SUB males. To investigate the functional role of *sst1.1* in regulating social behavior, we used CRISPR/Cas9 gene editing. In fish injected with the CRISPR/Cas9 genetic construct that possessed a mosaic of indels ("crispants"), aggression was significantly reduced compared to vehicle-injected sibling controls. We are currently breeding heterozygous mutants with frameshift alleles to establish homozygous null *A. burtoni.* Collectively, these findings will enhance our understanding of SST and how it may represent a novel mechanism through which social status is modulated in *A. burtoni*.

Disclosures: L.A. Saenz: None. **S. Young:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

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Program #/Poster #: PSTR399.06/Y5

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R03 MH134181

Title: Effects of social dominance on glutamatergic and GABAergic activity in the infralimbic cortex

Authors: *A. F. RADFORD, M. A. COOPER;
Psychology, Univ. of Tennessee, Knoxville, TN

Abstract: Neural activity in the infralimbic cortex (IL) is known to regulate responses to stress. Social defeat stress has been shown to increase IL c-Fos expression in dominant Syrian hamsters,

who display less stress-related behavior compared to their subordinate counterparts. However, it is unknown which specific behaviors during social defeat exposure elicit greater IL activity and attenuate stress-related anxiety. In this study, we used fiber photometry to measure *in vivo* calcium (Ca^{2+}) transients in IL glutamatergic and GABAergic cells in male and female hamsters during social defeat exposure and anxiety testing. We found elevated Ca^{2+} activity in IL GABAergic neurons when dominant males received an attack and when they fought back during social defeat. In contrast, subordinate males showed reduced Ca^{2+} activity in IL GABAergic cells during both behaviors. In addition, dominant females showed a larger decrease in Ca^{2+} activity in IL glutamate cells compared to subordinate females when being attacked during social defeat exposure. These data were surprising because they suggest that proactive resistance to social defeat in dominant hamsters is associated with IL inhibition. Subordinate males also showed reduced Ca^{2+} activity in IL GABAergic cells when displaying social vigilance during anxiety testing. Meanwhile, subordinate females showed reduced Ca^{2+} activity in IL GABAergic cells when grooming during anxiety testing. These data demonstrate that subordinate hamsters show greater stress-induced changed in Ca^{2+} activity in IL GABAergic cells compared to their dominant counterparts, albeit in a sex-dependent manner. These findings indicate that specific types of stress-related behavior activate glutamatergic and GABAergic neurons in the IL and advance our understanding of the cortical circuits controlling proactive stress coping.

Disclosures: A.F. Radford: None. **M.A. Cooper:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

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Program #/Poster #: PSTR399.07/Y6

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: R03 MH134181

Title: Knockdown of androgen and estrogen alpha receptors in the posterior medial amygdala reveals sex differences in the neuroendocrine mechanisms controlling stress resilience

Authors: *B. KRIEG[1], M. A. COOPER[2], A. F. RADFORD[3], E. G. HALLERAN[3];
[1]Psychology, Univ. of Tennessee, Knoxville, Knoxville, TN; [2]Dept. of Psychology, Univ. of Tennessee Knoxville, Knoxville, TN; [3]Univ. of Tennessee, Knoxville, TN

Abstract: Social stress is a risk factor for stress-related mental illness and understanding the neurobiology of stress resilience is essential for identifying novel targets for improved treatments. The posterior medial amygdala (MeP) is a hormone-sensitive brain region in which activity of gonadal steroid hormone receptors have been shown to enhance stress resilience. Syrian hamsters respond to acute social defeat stress with a prolonged increase in anxiety-like behavior, as measured by a conditioned defeat (CD) test in males and a social avoidance test (SA) in females. We hypothesized that viral-mediated knockdown of androgen receptors (AR)

and estrogen receptor α (ERα) in the MeP would enhance anxiety-like behavior in male and female hamsters, respectively. We conducted stereotaxic surgery in both male and female Syrian hamsters and bilaterally injected AAV-AR-shRNA, AAV-ERα-shRNA, or AAV-scrambled-shRNA into the MeP. Animals were given two weeks to recover from surgery and handled daily to habituate them to human contact. Animals were exposed to a CD and SA test prior to social defeat stress. The CD test consisted of a 5-min social interaction with a juvenile animal in the subject's home cage, and the SA test consisted of a 10-min exposure to an unfamiliar adult animal in a novel arena and across a perforated barrier. Social defeat exposure consisted of 3, 5-minute aggressive encounters with a larger, same-sex aggressor. Following social defeat exposure, animals again received CD and SA tests. We found that knockdown of AR in the MeP increased the CD response in males, but did not alter anxiety-like behavior in females. In contrast, preliminary data suggest that knockdown of ERα in the MeP increases anxiety in a SA test in females. Investigation into the effects of MeP ERα knockdown in males is ongoing. These findings indicate that expression of AR and ERα in the MeP reduced defeat-induced anxiety responses in males and females, respectively. Overall, these findings highlight sex-specific neuroendocrine mechanisms that mediate stress resilience in the MeP and contribute to our understanding of how hormone treatments might be used to buffer the effects of traumatic stress.

Disclosures: B. Krieg: None. **M.A. Cooper:** None. **A.F. Radford:** None. **E.G. Halleran:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.08/Y7

Topic: G.03. Stress and the Brain

Title: Stress-induced sensitization: the effects of psychedelics on enhanced fear expression post-trauma in a novel context in mice

Authors: *S. R. OTTOFARO[1], J. L. MALTMAN[1], J. GONZÁLEZ-MAESO[2];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]Physiol. & Biophysics, Virginia Commonewealth Univ., Richmond, VA

Abstract: Stress sensitization, briefly defined as a heightened fear response to neutral stimuli following trauma, is a common yet poorly understood characteristic of neurological conditions such as post-traumatic stress disorder (PTSD) and panic disorders. Current treatments are limited and often ineffective due to gaps in knowledge surrounding the mechanisms of fear generalization. In this study, we confirm that acute stress exposure sensitizes mice to a neutral stimulus presented in a novel environment, and assess whether psychedelic drug intervention mitigates this maladaptive response. Adult male C57BL/6J mice were assigned to control or experimental groupsand underwent baseline testing, fear acquisition via foot shock (or no shock for controls), and fear expression in both the original context and in response to a neutral cue (a

tone) in a novel context, with contexts being established using lighting and scent cues to simulate a real-life scenario. Freezing behavior was recorded across sessions, with percent freezing time being the primary outcome measure. Mice exposed to an acute stressor showed a significant increase in freezing behavior in response to a neutral stimulus, indicating stress sensitization. These behaviors were linked to region-specific changes in gene expression as measured via qRT-PCR, particularly in the levels of Epidermal Growth Factor Receptor (EGFR) in the the frontal cortex. We hypothesize that drug-treated mice will exhibitreduced freezing behavior compared to untreated stressed mice,accompanied by mitigated changes in gene expression,suggesting a therapeutic mechanism for fear generalization. This research demonstrates that the damage caused by traumatic stress goes far beyond behavior and gives direction for advancing the knowledge and treatment of under-represented symptoms of panic-related disorders.

Disclosures: S.R. Ottofaro: None. **J.L. Maltman:** None. **J. González-Maeso:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.09/Y8

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: PAPIIT UNAM IT200125
Secihti CF-2023-G-243
NIMH, NIH ZIAMH002386

Title: Pacap-expressing neurons in the bed nucleus of the anterior commissure modulate threat-evoked escape involving learning and memory

Authors: *V. S. HERNANDEZ[1], L. E. EIDEN[2], L. ZHANG[1];
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Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide implicated in stress and defensive behavior, yet its role in learning and memory circuits is poorly understood. We identified a population of PACAP-expressing neurons in the bed nucleus of the anterior commissure (BAC) projecting to emotion- and cognition-related regions, including the medial amygdala, lateral septum, and hypothalamus. To examine their function, we designed a behavioral assay combining a looming visual stimulus with a maze containing a shelter. PACAP knockout mice showed significant delays in reaching the shelter compared to wild-type controls. Similarly, chemogenetic silencing of BAC neurons in PACAP-Cre mice using Cre-dependent Gi-DREADDs and CNO impaired performance. Selective PACAP deletion in BAC neurons (PACAP^flox/flox with AAV-Cre) recapitulated this deficit without neuronal silencing, indicating a specific role for PACAP signaling. Control groups (non-Cre or CNO-only) exhibited no impairments. Experiments used male and female C57BL/6J mice, with no sex differences

observed. To assess network effects, we quantified Fos expression 90 minutes post-testing in PACAP knockout mice, revealing reduced activity in the lateral septum, diagonal band of Broca, anterior hypothalamus, mammillary nuclei, central, medial, and cortical amygdala, and hippocampal CA3 and dentate gyrus. These results suggest that PACAP signaling in BAC neurons modulates defensive behavior memory and orchestrates activity across a distributed limbic network.

Disclosures: V.S. Hernandez: None. **L.E. Eiden:** None. **L. Zhang:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.10/Y9

Topic: G.03. Stress and the Brain

Support: NIMH R01MH60670

Title: Behavioral and molecular signatures of stress-enhanced fear learning in mice

Authors: *S. N. AYALA ROSARIO[1], T. LUGO[3], O. MORGAN[2], C. W. HAWK[2], M. S. FANSELOW[4], G. R. POE[5];
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Abstract: Post-traumatic stress disorder (PTSD) is characterized by heightened fear responses and impaired extinction learning. The Stress-Enhanced Fear Learning (SEFL) model recapitulates these features in rodents, producing long-lasting fear responses following an initial traumatic stressor. Here, we examined sex differences in SEFL-induced behavior and assessed ΔFosB, a stable transcription factor associated with chronic stress and neural plasticity, as a marker of persistent brain changes. Male and female C57BL/6J mice underwent SEFL, followed by cued fear conditioning, extinction, and extinction recall across distinct contexts to model trauma generalization and recovery. SEFL exposure led to significantly elevated freezing in both sexes during the induction phase. However, only SEFL males exhibited significantly higher freezing during fear conditioning ($p = 0.0084$), extinction recall ($p = 0.0063$), and during the pre-tone period of extinction ($p < 0.001$), suggesting a sex-specific vulnerability to generalized and persistent fear. In contrast, SEFL females showed freezing levels comparable to nSEFL controls across phases, indicating behavioral resilience. No differences in pre-tone freezing were observed during SEFL induction or conditioning, confirming similar baseline fear across groups. ΔFosB expression is being quantified in the medial prefrontal cortex, amygdala, hippocampus, nucleus accumbens, and locus coeruleus. ΔFosB expression profiles may reflect region-specific neural adaptations that underlie the observed behavioral differences. These findings identify male-specific susceptibility to fear generalization and extinction memory deficits in mice

following trauma, and suggest ΔFosB as a molecular indicator of enduring brain changes linked to stress-exposed behavioral phenotypes.

Disclosures: S.N. Ayala Rosario: None. **T. Lugo:** None. **O. Morgan:** None. **C.W. Hawk:** None. **M.S. Fanselow:** None. **G.R. Poe:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.11/Y10

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Activation of ventromedial hypothalamic to anterior hypothalamic area output triggers complex escape behavior

Authors: *A. MOHEAD, B. LAING;
BioMolecular Sci., Univ. of Mississippi, Oxford, MS

Abstract: To date there are no reports regarding the causal effect of ventromedial hypothalamic area to anterior hypothalamic area (VMH→AHA) stimulation on complex escape behavior and the consequences of this stimulation on circulating inflammatory factors. This is interesting given that recent work has demonstrated that neurons in the AHA respond to innate and learned threats in the environment, and that activation of glutamatergic VMH→AHA projections generate jumping and locomotor behavior. Here, we used channelrhodopsin2-mediated optogenetic manipulation of VMH→AHA output. We microinjected AAV9-hsyn-FLEX-ChR2 into the VMH of SF1-creor WT mice (n = 7-8 male mice per group, 7-10 female mice per group). Experimenters were blinded to the genotype of mice at the time of experimentation. We found that while in an empty open field apparatus, VMH→AHA stimulation triggers jumping behavior at calculated stimulation intensities beyond 2mW/mm^2. However, if a ladder is placed in the apparatus experimental mice will climb out while control mice will not. Importantly, following stimulation mice exhibit significantly greater time in the closed arms of an elevated plus maze compared to control mice which is indicative of a sustained anxiogenic state. In addition, using echocardiography we found that anesthetized mice exhibit a significant elevation of heart rate and cardiac output following stimulation compared to controls. Further, we found significantly elevated pro-inflammatory cytokine interleukin-6 (IL6) levels in the plasma of experimental mice compared to controls. Taken together, we demonstrate for the first time that VMH→AHA stimulation triggers complex escape, increases anxiety related behaviors, physiological arousal, and circulating inflammatory cytokines. These findings provide insight into how the AHA triggers physiological and behavioral changes that are relevant to threat responding.

Disclosures: A. Mohead: None. **B. Laing:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.12/Y11

Topic: G.03. Stress and the Brain

Support: Fapesp 22/06044-9
Fapesp 22/16318-9
Fapesp 17/12881-2
Fapesp 22/14359-0
Fapesp 24/10023-2

Title: Balancing reward and fear: Investigating the neural circuitry of survival

Authors: *J. M. IKEBARA[1,2], N. S. CANTERAS[2];
[1]Dept. of Neurobio. and Anat., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX;
[2]Dept. of Anat., Univ. of Sao Paulo, São Paulo, Brazil

Abstract: Introduction and objectives: The cuneiform nucleus (CUN), traditionally associated with locomotor activity. However, some studies have shown that CUN has strong bidirectional links with the dorsolateral periaqueductal gray (dlPAG) and shares a similar source of inputs involved in integrating information related to life-threatening events. Indeed, CUN opto-stimulation generates innate freezing and flight antipredator response in rats. In this way, employing track-tracing, optogenetics, and calcium imaging experiments, we investigated differential role of glutamatergic (Glu) and GABAergic (GABA) neurons within CUN in transgenic mice.Methods and Results: Vglut2-Ires-Cre and VGAT-cre mice were used for all experiments. A stereotaxic surgery was performed to inject a vector viral for the following experiments: Tracing AAV-DIO-EYFP; Optogenetics: AAV-DIO-ChR2-EYFP; Fiberphotometry: AAV-DIO-GCamp6s, into the CUN. Tract-tracing unveiled similar projections of Glu and GABA to the dorsolateral PAG, but divergence in their connections to the hypothalamus. Glutamatergic neurons projected to the ventro-medial hypothalamic nucleus, while GABAergic neurons targeted the anterior hypothalamic nucleus. Opto-stimulation during an open field generates freezing and flight. Real-time place preference (RTPP) opto-stimulation, used to evaluate if the stimulus is aversive, indicated that Glu activation prompted an aversive response, contrasting with the unaltered state observed in GABA stimulation. However, neither Glu nor GABA seemed implicated in the acquisition of learned fear responses, as evidenced by contextual place preference tests. Further insights emerged from fiber photometry data, revealing the nuanced roles of Glu and GABA in looming stimulus and predator exposure. Glutamatergic and GABAergic neurons exhibited heightened activity during looming perception and escape behavior in predator exposure, but GABAergic population is likely more related to exploratory behaviors. The procedures are approved by the Animal Ethics Committee.Conclusion: These findings suggest a complementary dynamic between the two cell populations within CUN, contributing to the overall response in threatening contexts.

Disclosures: J.M. Ikebara: None. **N.S. Canteras:** None.

Poster

PSTR399: Defensive Behavior, Aggression, and Fear

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR399.13/Y12

Topic: G.03. Stress and the Brain

Support: FAPESP 2022/14359-0
FAPESP 2025/08983-0

Title: The nucleus incertus and its role in regulating behavioral activation

Authors: *R. S. JORGE, N. S. CANTERAS;
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Abstract: The Nucleus Incertus (NI) is a structure in the brainstem located in the periventricular pontine region. It has been shown to play a role in arousal, locomotion, and memory. However, its specific role in regulating transitions between states of high and low behavioral activation is still not fully understood. This project aims to investigate whether NI activity correlates with behavioral activation and whether manipulating this activity can influence these behavioral states. We are developing behavioral paradigms to contrast high and low levels of behavioral activation in mice. In a preliminary set of experiments, we utilized a controlled meal-feeding protocol involving restricted access to food to model high activation. During this protocol, the animals displayed significant hyperlocomotion, increased average speed, frequent jumping, and extensive exploration, particularly before the scheduled availability of food. This behavioral pattern appears to indicate a proactive arousal state associated with behavioral readiness rather than passive stress or homeostatic hunger. As a complementary model for low activation, we are implementing a social defeat stress paradigm, which is expected to produce sustained reductions in locomotion, exploration, and motivational drive, reflecting passive behavioral states. After behavioral testing, we will process brain tissue for Fos immunolabeling to assess NI activation. Preliminary data suggest that Fos expression in NI neurons increases during high behavioral activation, indicating a potential relationship between NI activity and arousal-related behaviors. In addition, we have conducted anatomical mapping of NI efferents using viral tracers, revealing strong projections to the medial septum, supramammillary nucleus, interpeduncular nucleus, lateral preoptic area, lateral hypothalamus, and dorsal raphe - regions that play critical roles in arousal and motivation. Ongoing efforts will focus on mapping NI afferents, with the hypothesis that inputs from the medial prefrontal cortex and lateral habenula contribute to NI-driven regulation of behavioral states. Optogenetic experiments in Neuromedin B-Cre mice are currently underway to determine whether targeted activation or inhibition of NI neurons and their projections can bidirectionally modulate behavioral activation. Together, these preliminary findings lay the foundation for understanding how NI circuits influence arousal, motivation, and adaptive behavioral responses, providing potential insights into the neural mechanisms underlying motivational deficits observed in psychiatric disorders such as depression.

Disclosures: R.S. Jorge: None. **N.S. Canteras:** None.

Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

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Program #/Poster #: PSTR400.01/Y13

Topic: G.03. Stress and the Brain

Support: NIH grant MH108286
NIH grant HD105771

Title: Paternal stress alters germ cell function and embryonic development rates

Authors: *K. C. CREUTZBERG, A. JENG, N. A. LEU, T. L. BALE;
Dept. of Psychiatry, Univ. of Colorado, Aurora, CO

Abstract: Stress is a key regulator of human behavior and physiology and can lead to long-term health issues. We recently demonstrated a link between perceived stress and altered germ cell function in humans. Such lasting effects of stress on reproductive somatic cells have also been implicated in altered offspring neurodevelopment and adult stress responsivity in preclinical studies. In the epididymis, sperm undergo critical maturation facilitated by extracellular vesicles (EVs) secreted by epididymal epithelial cells (EECs). However, the mechanistic link between stress-induced germ cell changes and offspring development remains poorly understood. To mechanistically examine this, we used a transgenic chemogenetic mouse model to mimic the cellular effects of stress by increasing EEC intracellular calcium signaling thereby increasing sperm mitochondrial activation and EV secretion. We chronically activated caput EECs that express the Gq-coupled Designer Receptor Exclusively Activated by Designer Drugs (DREADD) receptor hM3Dq with clozapine-N-oxide (CNO). Males (WT or DREADD+, n=14) received CNO for 4 weeks and were either bred for litter outcomes or euthanized for sperm and mitochondrial analysis. Sperm was assessed with the Computer Assisted Sperm Analysis (CASA) system. Mitochondria were evaluated using transmission electron microscopy and SeaHorse. For reproductive outcomes, males were bred and litter size was analyzed at gestational day 12.5. Finally, for embryo culture, superovulated females were mated with males overnight, and oocytes were collected the next morning to assess fertilization rates and embryonic development. Chronic treatment with CNO induced changes in sperm mitochondrial signal intensity ($p=0.049$), suggesting increased ATP production. DREADD+ males exhibited enhanced reproductive function, as evidenced by increased fertilization rates ($p=0.019$) and larger litters ($p<0.001$), with no changes in total oocyte number ($p=0.76$) or litter sex-ratio ($p=0.27$). Additionally, embryos from DREADD+ males exhibited accelerated development. Here, we demonstrated that this mouse model replicates many facets of our human studies, suggesting that paternal stress affects sperm function and cargo, altering fertilization and embryo development rates with potential downstream consequences for neurodevelopment. This data

provides key insights into how paternal experiences may shape reproductive outcomes and ultimately affect neurodevelopment.

Disclosures: K.C. Creutzberg: None. **A. Jeng:** None. **N.A. Leu:** None. **T.L. Bale:** None.

Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.02/Y14

Topic: G.03. Stress and the Brain

Support: NIMH MH108286
NIMH MH129495
NICHD HD097093
NICHD HD105771

Title: Human stress-associated sperm microRNA, let-7f-5p, alters embryo development

Authors: *A. JENG, K. C. CREUTZBERG, N. R. MOON, A. S. FELTRIN, N. A. LEU, C. N. EPPERSON, T. L. BALE;
Psychiatry, Univ. of Colorado Anschutz Med. Campus, Denver, CO

Abstract: Paternal lifetime exposures to environmental challenges are associated with increased offspring neuropsychiatric disease risk. Small non-coding RNA (sncRNA) are potential causal agents in the germline transmission of paternal experience, including trauma and stress, at conception. In a previous longitudinal, repeated sampling human cohort, we identified sperm microRNA (miRNA) that were significantly increased in response to elevated prior perceived stress. To understand the potential role of sperm let-7f-5p in the transmission of stress and its impact on embryo development, we investigated outcomes produced by increasing zygote levels of this miRNA. With mouse zygotic microinjections and RNA sequencing of let-7f-5p blastocysts, we found significant alterations in the developmental transcriptome, suggesting remodeling of both the inner cell mass and trophectoderm. To determine if these changes corresponded to alterations in developmental transitions, we utilized *in vivo* time-lapse imaging of these embryos from the zygote to blastocyst stage. RNA sequencing of fetal and placental tissues indicated changes in gene expression at mid-gestation (E12.5), where GSEA of fetal sequencing revealed enrichment of calcium regulation and ATP-dependent activity. Placental analyses revealed enrichment of cell cycle, translation, and RNA processing pathways. Notably, we found significant sex differences in the gene pathways impacted by let-7f-5p where only male placentas had significant upregulation of genes related to glucocorticoid regulation and activity. In adult offspring, only let-7f-5p males showed significantly increased body weights and long bone growth. Current studies are examining the impact of changes to embryo development rates on the adult hypothalamus as the key regulator of growth and metabolism. Given the potential for sncRNA to convey paternal life exposures, determining the effect of stress-responsive

miRNA on offspring neurodevelopment is essential to identify environmental risk and resilience factors.

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Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.03/Y15

Topic: G.03. Stress and the Brain

Support: DBT-Wellcome Trust India Alliance Intermediate Fellowship Grant IA/CPHI/18/1/503964)

Title: Epigenome-wide placental methylation patterns linked to maternal depressive symptoms in pregnancy

Authors: *T. CHINNASAMY[1], D. D[2], M. DEEPA[3], B. SRIKUMAR[4], U. RAM[6], A. RANJIT MOHAN[3], M. BALASUBRAMANYAM[3], V. MOHAN[3], P. SARAVANAN[7], K. GOKULAKRISHNAN[5];
[1]Neurochemistry, Natl. Inst. of Mental Hlth. and Neuro Sci., BANGALORE, India; [2]Yenepoya Reserach Ctr., Yenepoya Deemed to be Univ., Mangaluru, India; [3]Madras Diabetes Res. Fndn., Chennai, India; [4]Neurophysiol., Natl. Inst. of Mental Hlth. and Neuro Sci. (NIMHANS), BANGALORE, India; [5]Natl. Inst. of Mental Hlth. and Neuro Sci. (NIMHANS), Bengaluru, India; [6]Seethapathy Clin. & Hosp., Chennai, India; [7]Populations, Evidence and Technologies, Div. of Hlth. Sciences, Warwick Med. School, Univ. of Warwick, Coventry, United Kingdom

Abstract: Background and aim: Depressive symptoms during pregnancy have been linked to adverse health and developmental outcomes of the offspring. However, the biological mechanisms that mediate these long-term effects are not well defined. Emerging evidence indicates that maternal psychological stress during gestation may induce changes in placental DNA methylation, which could disrupt normal placental function and influence fetal growth and development. This study aimed to explore the genome-wide DNA methylation landscape of the placenta in relation to maternal depressive symptoms reported during early pregnancy. **Methods:** DNA methylation profiling was performed on 107 placental samples collected at birth from participants in the STratification of Risk of Diabetes in Early Pregnancy (STRiDE) cohort using the Illumina EPIC BeadChip array. Based on PHQ-9 scores during the OGTT visit at 24-28 weeks, women were categorised into those with (n=54) and without depressive symptoms (n=53). Epigenome-wide association study (EWAS) data analysis was performed with adjustments for maternal age, BMI, gestational age at delivery, gestational diabetes mellitus (GDM), parity, and infant sex. **Results:** Our results identified 8,986 differentially methylated CpG sites ($p < 0.01$) associated with maternal depressive symptoms. Pathway enrichment

analysis revealed involvement in estrogen signalling, thyroid hormone signalling, inositol phosphate metabolism, and axon guidance, each of which plays a critical role in fetal neurodevelopment and hormonal regulation. Interestingly, we identified the CpG site cg08789908 located in the gene 11β-hydroxysteroid dehydrogenase type 2 (*HSD11B2),* which was hypermethylated in women with depressive symptoms. Dysregulation of *HSD11B2* during prenatal development has been implicated in increased vulnerability to neuropsychiatric disorders, including depression, later in the offspring's life. **Conclusion:** Our findings provide mechanistic evidence that depressive symptoms during early pregnancy are associated with specific alterations in placental DNA methylation, particularly in genes and pathways implicated in neurodevelopmental and endocrine function deficits. Whether similar epigenetic signatures can be detected in offsprings of those mothers remains to be elucidated by further studies.

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Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.04/Y16

Topic: G.03. Stress and the Brain

Support: State University of New York (SUNY) Empire Innovation Program

Title: Maternal stress increases prenatal alcohol sensitivity and alters JAK/STAT activity

Authors: *T. DORMAN, K. BOSCHEN, H. SAWAL, S. FANG, K. BALACHANDRAN, O. HAN;
Neurosci. and Physiol., Upstate Med. Univ., Syracuse, NY

Abstract: Fetal Alcohol Spectrum Disorder (FASD) is associated with intellectual and behavioral impairments, craniofacial malformations, and growth deficits. However, prenatal alcohol exposure (PAE) does not impact all individuals equally, supporting investigation of other factors that may interact with PAE. This study uses a mouse model to examine whether maternal stress impacts fetal growth and whether co-exposure with alcohol modifies an offspring's susceptibility to developing PAE-related birth defects. Previous work found that the combination of stress and a binge-like dose of alcohol during early gestation result in high rates of litter loss and growth deficits. To determine the effect of stress alone on embryo development, this study assessed transcriptomics during gastrulation (embryonic day (E) 7 in mice, 3rd week of pregnancy in humans). This time point corresponds to when alcohol is administered. Pregnant female C57BL/6J mice were assigned to either standard group housing or maternal stress (isolation in a low bedding cage) across gestation (E0-E7). Embryos and extraembryonic tissue were collected on E7. The extraembryonic tissue was genotyped for sex. RNA was extracted

from each embryo for bulk RNA sequencing (n=4/sex/treatment). Genotyping was confirmed by comparison of Y-linked gene expression between the male and female samples. When assessing the impact of stress alone, stress was associated with upregulated genes involved in positive regulation of the JAK/STAT signaling pathway. Interestingly, 14 prolactin genes and 4 interleukin genes were upregulated and associated with this positive regulation. In addition, an upregulation in immune system processes and cytokine receptor binding was observed, also associated with prolactin genes, as well as nitric oxide synthase 2 (NOS2), cathepsin 7 (Cts7) and Serpinb9f. These genes are involved in inflammation and apoptosis. When observing the interaction of sex and stress exposure, a significant downregulation in Chac1, a gene involved in GSH degradation, was found in stress males vs. control males. Overall, this preliminary data suggests that stress during early gestation could upregulate the JAK/STAT signaling pathway, potentially through the upregulation of prolactin, resulting in an increase in expression of pro-inflammatory molecules. There may also be sex differences in the embryonic stress response. Given that alcohol can also upregulate the JAK/STAT pathway, resulting in inflammation, the combination of stress and PAE may result in additional JAK/STAT activation that could further exacerbate inflammatory responses and increase risk of fetal demise and growth deficits.

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Poster

PSTR400: Early Life Stress

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Program #/Poster #: PSTR400.05/Z1

Topic: G.03. Stress and the Brain

Support: NIH Grant MH135792
Loyola Undergraduate Research Opportunity Fellowships

Title: Sex-specific behavioral and synaptic differences following prenatal stress in mice

Authors: *T. BUCK[1], M. MCCARTHY[1], R. SOBREPENA[1], C. LEWIS[1], O. TAIWO[1], D. J. RADEMACHER[2], M. S. SODHI[1];
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Abstract: Prenatal stress (PRS) is linked to an increased risk of neurodevelopmental disorders, with distinct outcomes based on sex. Previous studies suggest males are more susceptible to social and cognitive deficits, while females show greater vulnerability to depression and anxiety. To explore this, we used a mouse model to investigate whether prenatal stress induces sex-specific behavioral deficits driven by synaptic differences. Pregnant Swiss Webster mice were exposed to 30-minute restraint stress twice daily during weeks 2 and 3 of gestation. At ten weeks

of age, PRS offspring (n=6 males, 5 females) and non-stressed (NS) controls (n=6 males, 6 females) were assessed for cognitive and social deficits through a battery of tests: Y-maze spontaneous alternation (SA), open field test (OFT), novel object recognition (NOR), three-chamber test of social approach (SOC) and social memory (SM), and contextual fear conditioning. PRS significantly increased contextual fear ($F_{1,13}$=10.60, $p<0.01$) in both sexes, but did not affect cued memory. Neither spontaneous alternation nor NOR performance was altered in any group. In females, PRS increased locomotor activity in the OFT ($p<0.05$), but had no impact on OFT risk aversion. PRS males, but not females, showed reduced social approach (SOC) behavior ($p<0.1$), while social memory was unaffected across groups. These findings suggest that contextual fear is sensitive to PRS, with pathology in the dorsal CA1 region, linked to fear behavior, showing fewer sex differences than the dorsal CA2, which is involved in social behavior. We are increasing the number of subjects in ongoing work to achieve optimal statistical power, in addition to performing analyses of synaptic alterations in the hippocampus and other brain regions through immunohistochemistry (IHC) of AMPA-type glutamate receptor (AMPAR) subunits. We are also analyzing synaptic morphology of 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate-stained dendrites using Filament Tracer software (Imaris) to explore the molecular and structural consequences of PRS in a sex-specific context. These insights could lead to more targeted therapeutic strategies for neurodevelopmental disorders.

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Poster

PSTR400: Early Life Stress

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Program #/Poster #: PSTR400.06/Z2

Topic: G.03. Stress and the Brain

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Title: Effects of lifelong low social status on adult prefrontal-amygdala-hippocampal circuit structure and connectivity: role of stress, neurotoxicity and cognitive outcomes in Macaques

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Abstract: Chronic social stress, especially starting early in life, is a risk factor for physical and mental illness, and cognitive decline during aging. To examine the biological and neuropathological mechanisms underlying lifelong effects of low social status we used neuroimaging tools to measure adult brain structure and connectivity in circuits critical for cognitive and stress regulation as well as markers of neurotoxicity (kynurenine pathway -KP-metabolites) in a translational rhesus monkey model of social subordination stress. Structural and resting-state brain MRI scans were collected in 27 adult female rhesus monkeys (13 dominant -DOM-, 14 subordinate -SUB-), aged 7-11 years, to measure volume of and functional connectivity (FC) between the hippocampus (HIPPO), amygdala (AMY) and prefrontal (PFC) subregions mediating cognitive flexibility (dorsolateral PFC(dlPFC) -Areas 9,46-; orbitofrontal cortex (OFC) -Area 13-; medial PFC (mPFC) -Areas 14, 24-ACC-, 25). CSF KP metabolites were measured as indices of neuroprotection versus neurotoxicity. Cognitive flexibility was measured with the Intra-/Extra-Dimensional (ID/ED) attention set shifting task. We examined the effects of social rank on MRI and cognitive measures using ANOVA models and used Spearman Rank correlations to assess associations between cognitive and MRI measures. SUB animals 1) had larger OFC (Area13: $F(1,23)=5.905$, $p=0.023$), mPFC (Area25: $F(1,24)=4.964$, $p=0.036$) and HIPP ($F(1,24)=6.034$, $p=0.022$), than DOM (particularly in the rostral right HIPP) and correlations suggested that bigger HIPP volume was associated with elevated levels of the stress hormone cortisol ($rho=0.452$, $p=0.04$); 2) had lower levels of the neuroprotective metabolite kynurenic acid (KYNA, $rho=0.416$, $p=0.035$); and 3) made more errors in ID shifts ($rho=0.512$, $p=0.06$). Larger OFC, mPFC and AMY volumes were associated with increased Reversals 2 and 3 errors (e.g. Area13_L vs Rev3: $rho=0.631$, $p=0.016$; Area24_Total vs Rev2: $rho=0.745$, $p=0.002$), whereas larger HIPP volumes predicted more ID errors ($rho=0.525$, $p=0.054$). Individual differences in FC in PFC-AMY-HIPP circuit predicted the number of reversal and ID errors in the cognitive task. In summary, social subordination stress leads to structural and functional alterations in PFC-AMY-HIPP circuits in adulthood associated with neuropathological changes and mild impairments in cognitive flexibility, i.e. behavioral inhibition (reversal errors) and shifting attention (ID errors). We will study these animals at later ages to identify whether the cumulative social stress experienced by SUB animals accelerates their biological and neurocognitive aging.

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Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.07/Z3

Topic: G.03. Stress and the Brain

Support: Postdoctoral fellowship from the George E. Hewitt Foundation for Medical Research

Title: Effects of early life adversities on development and maturation of hippocampal network dynamics and memory function

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Abstract: Background: Experiencing early-life adversity (ELA), such as chaotic environments, is a potent risk factor for persistent cognitive deficits, particularly memory impairments. While human epidemiological studies previously showed a strong link between ELA and poor cognitive outcome, the underlying mechanisms by which ELA disrupts the maturation of hippocampal-dependent memory systems during early postnatal development remain poorly understood. So far, it is known that the functional maturation of the hippocampus is characterized by a sequence of neuronal activities, which are initially intrinsically generated but modulated by sensory inputs from the environment to allow the development of mature network rhythms required for complex memory processes. In this project we investigate the novel hypothesis that ELA interferes with these early organizing network activities resulting in aberrant brain circuit formation and life-long memory problems. **Methods:** We use a validated rodent model of ELA involving limited bedding and nesting material from postnatal days 2-10, which generates chronic stress in the pups due to chaotic and unpredictable maternal sensory signals, and reliably induces memory deficits in adult mice. High resolution *in vivo* electrophysiology is employed to monitor hippocampal activity of pups with and without exposure to ELA and establish the effects of patterned vs unpredictable sensory input on network dynamics in neonatal mice. Based on the results obtained, we will then assess whether simulating physiological activity replicating endogenous sharp waves via minimally invasive transcranial optogenetic stimulation of pyramidal neurons allows to restore network maturation in ELA pups by characterizing hippocampal rhythms at multiple developmental stages. To assess the translational potential of this approach, it is then tested whether administrating physiological stimulation during ELA is sufficient to rescue hippocampus-dependent memory functions long-term by subjecting adult ELA mice with and without intervention to a battery of behavioural tests. **Conclusion**: This work combines advanced developmental electrophysiology recordings with non-invasive neural stimulation to uncover the mechanisms linking the experience of ELA with persistent memory deficits in adulthood. If causality of disrupted early network activity for cognitive dysfunction is confirmed and can be restored using optogenetic stimulation, this project would have broad implications for preventing cognitive disorders rooted in early-life stress. **Funding**: This work is supported by the George E. Hewitt Foundation for Medical Research.

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Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.08/Z4

Topic: G.03. Stress and the Brain

Support: CONAHCYT (252808) to Gonzalo Flores

Title: Post-weaning social isolation impairs dendritic spine plasticity in the rat hippocampus

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Abstract: Chronic stress is a well-established risk factor for several psychiatric disorders. The brain is particularly susceptible to the physiological effects of chronic stress, especially during critical periods of development when these effects can be more pronounced. Early-life chronic stress, in particular, has been implicated as a major contributor to the development of major depressive disorder (MDD). In this study, we investigated the effects of post-weaning social isolation on open-field and social behaviors, recognition memory (assessed using the novel object recognition test, NORT), and dendritic spine plasticity in dorsal hippocampal CA1 pyramidal neurons of adult male and female rats. Socially isolated males exhibited increased locomotor activity in the open field, an effect not observed in females. Recognition memory was impaired in both sexes, as isolated rats did not show an increased recognition index during either the short-term or long-term phases of the NORT. Dendritic spine density in the distal basal dendrites of CA1 pyramidal neurons was reduced in both isolated males and females. However, a significant decrease in mushroom spine density was observed only in isolated males. These findings emphasize the negative impact of early-life chronic stress on dendritic spine plasticity, which may represent a promising target for novel antidepressant therapies. The results also highlight sex-specific differences in behavioral and neural outcomes, which could influence individual responses to antidepressant treatment.

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

Support: Intramural Programs of the National Institutes of Mental Health (V.A.A.: ZIAMH002987; C.R.G.: ZIAMH002497)

Title: Early life adversity interacts with alcohol exposure and sex to preferentially increase striatal dopamine D1/D2 receptor ratio and social drinking in male mice

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Abstract: Adverse experiences early in life can change how neural circuits develop and respond to reward in adulthood. Early life adversity (ELA) is known to robustly and dose-dependently confer vulnerability to both mood and substance use disorders (SUDs), including alcohol use disorder (AUD). Yet, adverse experiences in childhood do not invariably lead to SUD development. Some hypothesize that other factors—including sex, experiences later in life, and the development of comorbid mood disorders—mediate the effects of ELA. Neurons within the brain's reward circuitry undergo protracted development through adolescence, making them a key target for studying ELA-induced changes to reward processing. In this study, we investigated whether ELA induced by a Limited Bedding and Nesting (LBN) paradigm was sufficient to alter risk avoidance, sensitivity to the anxiolytic effects of alcohol, and AUD-like behaviors in C57BL6/J mice, and whether alterations to dopamine D1- and D2-receptor balance in the striatum could underlie these behaviors. We found that adult mice (PND 60) exposed to LBN-rearing from PND 3-10 showed increased risk avoidance in a light-dark box ($t = 2.97$, $p = 0.0034$, t-test) and open field test ($t = 2.47$, $p = 0.015$, t-test) and voluntary consumed more alcohol in a self-paced, operant social drinking task ($F(1, 95) = 8.58$, $p = 0.0040$, ANOVA, $n = 175$). Differences in alcohol consumption were especially pronounced among male LBN-reared mice ($F(1, 49) = 6.69$, $p = 0.013$, ANOVA), as is consistent with past findings (Okhuarobo et al., 2020). Sensitivity to alcohol's anxiolytic effects was similar in LBN-reared and control mice of both sexes ($F(1, 96) = 1.01$, $p = 0.32$, ANOVA). Quantification of Drd1 and Drd2 relative expression via qPCR in bulk nucleus accumbens and dorsomedial striatum tissue identified one potential neurobiological mechanism connecting alcohol drinking and ELA and pointed toward an interaction with alcohol exposure and sex at the post-transcriptional level. Male LBN-reared mice showed a significantly higher ratio of Drd1:Drd2 expression than control males in the dorsomedial striatum ($p = 0.038$, t-test) and NAc ($p = 0.057$, t-test), but only after alcohol exposure. Autoradiography analysis of D1- and D2-like receptor binding confirmed that striatal receptor balance remained altered after translation. In all, our results point toward interacting influences of sex and alcohol exposure on the effects of early life adversity on striatal reward circuitry and identify a relevant neurobiological mechanism that may explain the compound effect of early life adversity and alcohol drinking on brain physiology and behavior.

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

Support: NIH Grant R01DA049837
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Title: Early Resource Alters Neurovasculature in the Medial Prefrontal Cortex of Female but not Male Rats

Authors: *L. BERHANE, E. ANDREWS, A. SILVA, L. HOOT, D. BANGASSER; Neurosci., Georgia State Univ., Atlanta, GA

Abstract: Early life adversities, such as resource scarcity, can induce stress that can reprogram brain development. In rats, we model adverse environmental experiences using a limited bedding and nesting (LBN) paradigm, in which dams and offspring experience resource deprivation from pups' postnatal day 2 to 9. LBN offspring are compared to control rats raised in standard caging with ample bedding and nesting. Our lab has previously shown that LBN causes transcriptional changes in the neurovasculature of the prefrontal cortex (mPFC) in adult female but not male rats. In this study, we expand on this work to determine whether these transcriptional changes result in changes in the vessels and whether there are sex differences in this effect. Fixed mPFC tissue was collected from male and female, control and LBN rats, and then sectioned. We performed immunohistochemistry for astrocyte end feet with an aquaporin 4 (AQP4) antibody and for endothelial cells that line vessels with a CD31 antibody. Sections were then imaged using confocal microscopy and quantified for average fluorescent intensities in Fiji. We found that AQP4 and CD31 were less intense in LBN vs. control females. There was no difference in the males. Adult chronic stress can cause astrocyte end feet to pull away from blood vessels, so we took a ratio of AQP4:CD31 intensity but found no difference. Collectively, these findings suggest that LBN reduces blood vessels in females, but this developmental stressor, unlike adult stress, does not cause astrocyte end feet retraction. Neurovasculature develops postnatally, and these findings suggest that it may be an important, yet underexplored, mediator of the lasting effects of the early environment on adult brain function

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

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Beckman Institute at Caltech

Title: Early Life Adversity and Naturalistic Threat Perturb Prefrontal Cortical and Limbic Network Dynamics

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Abstract: Neurophysiological consequences of early life adversity (ELA) in adulthood remain an open question. Neuroimaging coupled with computational analysis enables mapping of brain states - widely distributed, overlapping networks of neural activity. Such approaches promise to deliver insight into changes in adult brain states after ELA. We use Mn(II)-enhanced MRI (MEMRI) to label active neurons, which can be imaged longitudinally across successive conditions in freely moving animals. In this report, we applied structural equation modeling (SEM) to assess the effects of ELA and acute threat on functional connections based on MEMRI data. MEMRI data was acquired from 10-week-old mice reared under either standard or limited bedding conditions (n=24). Mice underwent 3D MRI at 100 μm^3 isotropic resolution at four timepoints: before and 22 h after $MnCl_2$ IP injection (0.3 mmol/kg); immediately and at 9 days after exposure to a naturalistic acute threat (TMT). To perform SEM, we employed the lavaan R package. SEM tests whether brain regions are connected based on whether covariance of signal intensities between locations within a hypothetical circuit across individuals fit a predicted model. A good fit is determined by obtaining $\chi^2_{pvalue} > 0.05$, CFI > 0.9, and SRMR < 0.08. First, to verify our computation, we replicated SEM results from a UCLA workshop. To test whether covariance of interregional signal intensities was a meaningful metric for circuit coactivations, we applied SEM to the well-characterized olfactory circuit, and found a good fit from our MEMRI data. To further test the ability of SEM to detect biologically meaningful covariance, we tested the fit of our data to models that were unconnected, randomly connected, or with randomized connections, as negative controls. SEM of reward and defensive circuitry models identified disrupted neural dynamics in ELA compared to Standard mice. We further examined functional connections among limbic system targets of projections from infra/pre-limbic or anterior cingulate cortex, previously reported in our stereotactic MEMRI tract tracing papers (Bearer and Jacobs). SEM revealed multiple, unique and overlapping, subcircuit coactivations within these two prefrontal cortical circuits that yielded a good fit after threat. These results are based on hypothetical circuits for which regions and connections may not be selected accurately and are dependent on the resolution of our anatomical segmentation. Our findings indicate distinct states within emotional regulation circuits that are shaped by ELA and further modulated by subsequent threat exposure.

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

Support: NIMH R01MH119164
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Title: Environmental enrichment rescues deficits in reversal learning and perineuronal net formation in the orbitofrontal cortex of adult mice exposed to early life adversity

Authors: *L. GIULIANO, S. JAMWAL, T. PASAM, R. ISLAM, Z. A. MACDOWELL KASWAN, S. AHMED, C. BOWERS, A. KAFFMAN;
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Abstract: Early life adversity (ELA) is a significant contributor to long-term cognitive impairments and structural changes in the brain. Individuals who were neglected and deprived during childhood have shown neural and behavioral changes such as hyperactivity, cortical thinning, and deficits in cognitive flexibility. Although neglect is one of the most common forms of ELA, these results have yet to be reproduced in a rodent model and have not been reversed using environmental enrichment (EE). We have recently replicated these findings in mice by providing them with limited bedding (LB) in their cages to simulate impoverished conditions. In this study, we examined EE as a potential mitigator of ELA's negative effects by giving LB mice access to toys in their cages during postnatal day 14 (P14) to P25. We used a Barnes Maze paradigm to assess reversal learning in adulthood, which was impaired in LB mice. We found that EE completely reversed this deficit. LB mice that exhibited reversal learning deficits also showed a reduction in c-Fos activation in the orbitofrontal cortex (OFC). We also found significantly less parvalbumin (PV)-positive cells in the OFC that were surrounded by perineuronal nets (PNNs) in LB mice, as well as an increase in the excitatory-inhibitory (E-I) synapse ratio. All of these abnormalities were reversed by providing EE to LB mice. Additionally, we used stereotactic surgery to introduce a PNN-degrading enzyme into the OFC of adult mice, which led to similar deficits in reversal learning, decreased PV-PNN colocalization, and an increased E-I ratio as observed in LB mice, suggesting that PNNs play a crucial role in maintaining adaptive neural and cognitive function in the OFC. To elucidate possible mechanisms underlying the observed reduction of PNNs and their colocalization with PV-positive cells in LB mice, we are now examining the expression of orthodenticle homeobox 2 (Otx2), a key regulator of PNN formation, in the OFC as well as in the choroid plexus (CP) where this transcription factor is synthesized. If Otx2 is confirmed to be involved in the deficits observed in adult LB mice and recovery of these deficits by EE, this work will reveal a novel mechanism explaining how deprivation and enrichment can coordinate cognitive flexibility in adulthood. This would also suggest the potential use of Otx2 administration as a treatment for enhancing cognitive flexibility in a mouse model of neglect. Together, these findings highlight ELA's profound impact on the ability to remain cognitively flexible throughout adulthood and identify EE as a possible intervention for preventing damage caused by childhood deprivation.

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

Support: Research Council of Finland #326045

Title: Early life stress induces male-specific changes in the spread of neuronal avalanches in the prefrontal-amygdala circuitry

Authors: *Z. KHARYBINA[1], S. PALVA[2], J. M. PALVA[4], S. E. LAURI[2], H. HARTUNG[2,3], T. P. TAIRA[1,3];
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Abstract: Early life stress (ELS) takes a significant toll on mental development, being a known risk factor for emotional, behavioral, and cognitive impairments. Neuronal circuits responsible for emotional processing, namely basolateral amygdala (BLA) and prefrontal cortex (PFC), are particularly susceptible to ELS. Given ELS-induced multifaceted anatomical and functional changes in the abovementioned regions, the current study aims to investigate how these changes affect network dynamics in the prefrontal-amygdala circuitry in terms of neuronal avalanches. We induced ELS in Wister rat pups using maternal separation (MS) protocol with pups being daily separated from the dam for 3 hours during postnatal days (p) 2 - 14. Using in vivo multielectrode recordings on urethane-anesthetized animals we assessed neuronal activity at two developmental stages, juvenescence (p 14 - p 15) and young adulthood (p 50 - p 60). In both PFC and BLA neuronal activity (estimated as negative deflections of local field potentials, or nLFPs) clustered together forming neuronal avalanches. To assess activity spread in the avalanches we calculated branching ratio, a parameter that estimates how many events (nLFPs) on average are triggered by a single event. In juvenile animals we failed to see any MS-related differences in the branching ratio in both regions, whereas in the adulthood for a large set of parameters used for avalanche detection MS group was characterized by an increase in the branching ratio in both PFC and BLA compared to the control group. Interestingly, the effect was significant only in males but not females. Next, we assessed activity spread between the regions. While most of the avalanches were restricted to one region, 10% and 5% of nLFP clusters (for juvenile and adult animals, respectively) happened simultaneously in both regions. For the avalanches starting in PFC and followed by nLFP clusters in BLA we saw an increased prevalence of larger avalanches in MS group. In addition, on average each event in PFC was followed by a smaller number of events in BLA compared to the control group. These effects were seen only in the adult males.

This could reflect previously shown MS-induced male-specific accelerated maturation of negative connectivity from PFC to BLA. MS had no effect on the avalanches starting in BLA, indicating unaffected spread of activity from BLA to PFC. Our findings show for the first time that ELS has a profound male-specific effect on neuronal avalanches in the prefrontal-amygdala circuitry, leading to intensified activity spread within both PFC and BLA, and impaired information transfer from PFC to BLA in young adult male rats.

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

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Title: Early Life Pain Alters Cellular Phenotypes of the Developing Amygdala and Hypothalamus

Authors: *B. M. MERRILL[1,2], M. E. TOMASCH[1,2], M. A. BURMAN[3];
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Abstract: Time spent in the neonatal intensive care unit (NICU) often increases one's susceptibility to anxiety and chronic pain conditions. We use a rodent model of a NICU-like experience, through which we have previously observed enhanced expression of corticotropin releasing hormone (*crh*) cell populations acutely following pain, with significant long-term reductions of *crh*+ cells in the right central amygdala (CeA) of adolescent male rats who experienced early life pain. The CeA contains a heterogeneous population of cells that express various biomarkers (e.g., *crh*, prodynorphin [*pdyn*], somatostatin [*sst*]), which have distinct functions in pain, fear, and anxiety. Research often fails to consider that cells co-express multiple neurotransmitters with distinct functional roles, especially across development and in response to pain. While there is a body of work which indicates *crh*+ cells may also co-express other pro- or anti-pain biomarkers like *pdyn* or *sst*, this project is innovative in examining how biomarker expression changes across development, in multiple brain regions following an early-life pain and stress model. We hypothesized that early life pain alters the composition of biomarker-identifiable subpopulations (e.g., *crh*, *pdyn*, *sst*) within the CeA, basolateral amygdala, paraventricular nucleus, and ventromedial hypothalamus, via a trauma-induced neural plasticity

that primes for altered pain responses and anxiety-like behaviors in later life. Rats in our NICU-like environment experience hind paw pricks four times a day, every two hours, for the first week of life. On post-natal day (PD) 12, 24, and 48, brain tissue was collected from male and female rats that experienced neonatal pain or were left undisturbed. To assess changes in cellular phenotype of the amygdala and hypothalamus across development, we performed RNAscope® fluorescent in situ hybridization to visualize *crh*, *pdyn*, and *sst* with slide scanner imaging. We used QuPath bioimage software to quantify cells (co-)labeled for these biomarkers. Following outlier testing and exclusion, we performed ANOVAs via SPSS, within each region of interest. Among our twelve experimental groups (n = 4-6/group) that account for condition (undisturbed, NICU), sex (male, female), and age (PD 12, 24, 48), we found age-dependent changes in the number, percentage, and overall developmental trajectory of cells labeled or co-labeled for *crh*, *pdyn*, and *sst*. Therefore, data suggest that early life pain may lead to sex-dependent changes in developmental trajectory of *crh*, *pdyn* and *sst*, and altered cellular specialization, which could account for changes in emotional and pain behaviors later in life.

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Poster

PSTR400: Early Life Stress

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Topic: G.03. Stress and the Brain

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Title: Alterations of Cognitive Behaviours and Prefronto-Thalamic Circuits after Early-Life Exposure to Fluoxetine

Authors: *N. N. SOTO[1], A. DE STASI[2], N. KARALIS[3,1], P. GASPAR[4], A. BACCI[5];
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Abstract: In the study of depression, emotional alterations have been the focus of the attempts to understand the disorder. However, other symptoms include neurovegetative changes and distinct cognitive impairments. Among other structures, the prefrontal cortex (PFC) plays a significant role in the regulation of higher order cognitive functions and emotional processes, and it is involved in the pathogenesis of depressive disorders. These regulatory and pathological roles, specifically in the context of cognitive control, result from robust reciprocal interactions with the mediodorsal nucleus of the thalamus (MD), among other regions. A critical developmental stage of the PFC occurs during the first 2 postnatal weeks, when a specific subtype of deep-layer pyramidal neurons (PNs) transiently expresses the serotonin transporter (SERT). In this

developmental period, the PFC is highly sensitive to environmental stressors including exposure to selective serotonin reuptake inhibitors (SSRIs) like fluoxetine (FLX). Perinatal FLX treatment (PNFLX) in rodents results in PFC hypoexcitability, altered SERT+ PN firing, and impaired emotional behaviors in adulthood. In addition, SERT+ PNs exhibit a dense bidirectional connectivity with the MD. Here, we used anatomical labeling, in vivo and in vitro electrophysiology, and a battery of cognitive tests to dissect the effects of PNFLX on the prefronto-thalamic loop and cognitive functions. We found that PNFLX results in cognitive impairments, including novelty aversion in the novel object recognition task, and sex-specific deficits in learning and attentional shifting in a cognitive flexibility task that is heavily dependent on the prefronto-thalamic loop. We then optogenetically mapped reciprocal connections between the PFC and MD by expressing the light-sensitive opsin ChR2 in either PFC SERT+ PNs or thalamocortical neurons of the MD. PNFLX resulted in a significant reduction of glutamatergic transmission from SERT+ PNs onto MD neurons. Conversely, MD glutamatergic recruitment of L2/3 PNs was significantly increased by PNFLX. Currently, we are using high-density electrophysiological approaches in vivo to assess global prefronto-thalamic network activity after PNFLX, aiming to unravel the circuit's pathophysiology underlying the behavioural impairments. Our results indicate that alterations of 5-HT levels during early development induces cognitive deficits in addition to emotional depressive-like symptoms in mice. The bi-directional synaptic alterations of the prefronto-thalamic pathway may explain in part the cognitive behavioral impairments observed in our developmental model of depression.

Disclosures: N.N. Soto: None. **A. De Stasi:** None. **N. Karalis:** None. **P. Gaspar:** None. **A. Bacci:** None.

Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.16/Z12

Topic: G.03. Stress and the Brain

Support: NIH NIGMS P20GM103423

Title: Enduring effects of ketamine treatment are influenced by dose, sex, and cell specific mechanisms across two models of affective dysfunction

Authors: A. LEMESHOVA, K. PATEL, *J. A. HONEYCUTT;
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Abstract: Affective dysfunction is associated with a myriad of psychological disorders - including depression, anxiety, and post-traumatic stress disorder - and significantly impacts quality of life with clear differences in sex/gender prevalence. While many patients benefit from typical antidepressants to treat symptoms, there remain a substantial number of people whose symptoms show treatment resistance. Studies have recently shown that ketamine, a non-

competitive NMDA receptor antagonist, shows promise in patients with treatment resistance both acutely and for an extended period following acute ketamine administration. However, there is debate as to the mechanism through which ketamine has its therapeutic effects, as well as how sex and risk type (i.e., genetic predisposition versus environmental stress) may impact ketamine's efficacy on brain and behavior. To investigate this, we used two models of affective dysfunction: the Wistar Kyoto (WKY) rat strain (genetic predisposition) and a maternal separation early life adversity (ELA) rat model. Rats received a single dose of ketamine (5, 10, or 15mg/kg IP) before behavioral testing 1 and/or 7 days post treatment. Rats were tested for acoustic startle response or anxiety-like behavior in the open field when presented with a threat cue (22 kHz alarm call playback). Our results suggest that female rats show improved behavioral outcomes in both assays when given 10 or 15mg/kg ketamine, with results persisting up to one week post-treatment. We investigated putative neural changes that might explain ketamine's actions. Our group has shown that ELA impacts parvalbumin (PV)-containing inhibitory neurons in the prefrontal cortex (PFC) in a sex-specific manner via changes in PV cell count, as well as DNA methylation levels within colocalized PV cells, which is reversed by ketamine in female rats. Here, we sought to determine the extent to which changes to PV-specific DNA methylation may be driven by oxidative stress and/or alterations to perineuronal nets surrounding PV cells and their relationship to behavioral outcomes in response to potential threat. We present data showing sex-specific effects of ketamine on perineuronal nets, DNA methylation (via 5-methylcytosine), and oxidative stress (via 8-Oxo-2'-deoxyguanosine specific to PV neurons within the PFC. Our findings suggest that higher doses of ketamine, particularly in females, show both acute and enduring effects on both behavior and neural outcomes across both ELA and WKY models. Thus, PV neurons are likely targets for ketamine's sex-specific effects, especially when considering pathologies that show dysfunction with threat assessment.

Disclosures: A. Lemeshova: None. **K. Patel:** None. **J.A. Honeycutt:** None.

Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.17/AA1

Topic: G.03. Stress and the Brain

Support: EJEP-5 PhD. Scholarship

Title: Therapeutic potential of heat-killed Enterococcus faecalis (EC-12) Para-Probiotic in reversing maternal separation-triggered social deficit in Mice

Authors: *E. A. MADY[1,2], H. M. EL-HUSSEINY[1,4,3], Y. MAKIOKA-ITAYA[5], R. INOUE[6], Y. YAMAMOTO[1], K. NAGAOKA[1];
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Univ. of Agr. and Technol., Fuchu, Tokyo, Japan; [5]Life Sci. Division, Combi Corp., Saitam, Japan; [6]Lab. of Animal Science, Dept. of Applied Biol. Science, Setsunan Univ., Osaka, Japan

Abstract: Maternal separation (MS) stress in early life was established as a model of triggering lifelong mental disorders. It can disrupt the gut-brain axis (GBA) and induce neurobiological illnesses in the adult stage. Diverse probiotics were utilized to reverse the encountered alterations. However, the employment of heat-killed *Enterococcus faecalis* (EC-12) to reverse the psychological abnormalities following MS has not been investigated yet. Hence, we aim to study the effectiveness of EC-12 as a potential therapy for MS mice. A total of 24 mice were divided into two main groups (control and MS, (n=12/group)). MS was conducted on postnatal days (PD) 3-20, until weaning on PD 21. On PD 22, mice were subdivided into 4 groups with and without EC-12 supplementation (n=6/group). Following 5 consecutive weeks of EC-12 supplementation, mice were subjected to behavior testing and then euthanized for sample collection and molecular assessments. MS mice exhibited a reduction in social behavior, meanwhile, the addition of EC-12 could improve this effect (prolongated interaction time in the stranger mice zone in the MS-EC-12 group, p=0.0174). The gut microbiome was substantially altered among the different groups. Transcriptome biological enrichment analysis revealed that vasopressin secretion was significantly changed between the MS-EC-12 and the MS groups (p=0.002). Diverse previous investigations highlighted the correlation between vasopressin, social interaction, temperament, and emotional status in animals and humans. Also, vasopressin-regulating genes; *Trpv4* and *Ecrg4*, were significantly up-regulated in the MS-EC12 versus MS animals (p=0.0003, and 0.004, respectively). For serum metabolome, L-tryptophan, and L-Tyrosine were more abundant in the MS-EC-12 group when compared to MS group. Metabolome of the cecal contents revealed that phenylacetic acid, pectin, glycerol 3-phosphate, and glycerol were more abundant in the MS-EC-12 than the MS group. KEGG enrichment analysis identified that glycolipid metabolism was a significantly abundant pathway in the cecal metabolome between the MS and MS-EC-12 groups. Our study illustrates the potential use of heat-killed EC-12 as a novel Para-Probiotic that could reverse the social abnormalities induced at the adult stage by exposure to MS in early life.

Disclosures: E.A. Mady: None. **H.M. El-Husseiny:** None. **Y. Makioka-Itaya:** None. **R. Inoue:** None. **Y. Yamamoto:** None. **K. Nagaoka:** None.

Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.18/AA2

Topic: G.03. Stress and the Brain

Support: NIH / NIDA DA036533 (Skowron, PI)

Title: Effect of parent-child interaction therapy on neural correlates of cognition in children with a history of adverse childhood experiences

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Abstract: Adverse childhood experiences (ACEs) are highly prevalent, with approximately one in six children experiencing at least one traumatic event before adulthood. Individuals with exposure to ACEs often show alterations in behavioral, neural, and physiological outcomes that may interfere with healthy development and often require intervention to restore functioning and foster resilience. Parent-Child Interaction Therapy (PCIT) is a parent-training intervention for children with disruptive behavior problems that also benefits with families affected by ACEs, using personalized coaching to improve relationships. While its behavioral effectiveness is well established, investigations into its impact on upstream neural processes are an emerging area of research. Using event-related potential (ERPs) with dense array EEG during a dichotic listening task, the current study examined how ACEs, timing, gender, and fatigue moderated cognitive outcomes of PCIT in a large sample of parent-child dyads (N= 204). For children, linear mixed-effects models revealed consistent distinctions in ACE groups taking part in PCIT therapy, indicating that high-ACE individuals demonstrated significantly greater change in ERP amplitude from pre- to post-intervention compared to low-ACE individuals, who showed superior baseline performance but minimal change over time. These differential training effects were most pronounced for attended stimuli compared to unattended stimuli, which showed more complex treatment × gender interactions. High-ACE children who engaged in the PCIT behavioral parent training intervention demonstrated particularly robust gains, especially during later portions of attention tasks, suggesting enhanced sustained attention capacity. Gender moderated these effects, with high-ACE boys showing the greatest treatment responsivity. Results indicate that children exposed to higher levels of early adversity may have enhanced neuroplasticity during attention training, supporting the potential of resilience-oriented intervention approaches. These findings have important implications for personalizing family-based interventions, suggesting that ACE history should inform treatment specifics and expectations, with low-ACE children potentially requiring a slightly different approach than the high-ACE children due to their lack of change over the treatment period.

Disclosures: B. Arledge: None. **E. Skowron:** None. **D. Bard:** None. **L. Ethridge:** None.

Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.19/AA3

Topic: G.03. Stress and the Brain

Support: NINDS: F99NS139541 (AMC)
NIMH: R01MH116900 (IM)
Hope for Depression Research (EJN)

NIMH: R01MH129306 (EJN)
HHMI (IM)

Title: Histone H3 Serotonylation Regulates Developmental Gene Expression in the mPFC Contributing to Behavioral Responses to Early Life Adversity

Authors: *A. CUNNINGHAM[1], J. CHAN[2], E. BRINDLEY[4], L. HOLT[3], T. MARKOVIC[6], V. KONDEV[5], M. ESTILL[7], L. SHEN[7], E. J. NESTLER[8], I. S. MAZE[9];
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Abstract: The serotonergic (5HTergic) system and its homeostasis during early life are essential for establishing proper brain architecture. Exposures to early life stress (ELS) can disrupt this process, increasing an individual's lifetime risk for affective disorders. Beyond its roles in receptor-mediated signaling, 5HT can covalently bind to histone H3 tails, thereby establishing H3 serotonylation (H3 Ser.). While H3 Ser. regulates neuroplasticity *in cellulo*, its functions during postnatal development, as well as the impact of ELS on this modification, *in vivo* remains unclear. Here, we employed brain region- and cell-population-specific epigenomic and transcriptomic profiling to assess normal and ELS-induced molecular changes in both the dorsal raphe nucleus (DRN) and the medial prefrontal cortex (mPFC), an important 5HTergic projection region that is central to stress responsivity and behavioral regulation. We observed altered transcriptional patterning within the DRN in response to ELS, which contributes to reduced 5HTergic innervation and 5HT abundance in the mPFC. Using FANS-coupled CUT&RUN- and RNA-sequencing in male and female mice, we further characterized H3 Ser. enrichment across postnatal development and following ELS exposures. Developmental and ELS-induced differences in H3 Ser. Were found to be sex-specific and cell-type dependent, with the most pronounced effects observed in glia. In these non-neuronal cells, ELS was found to increase H3 Ser. differential enrichment by over 150-fold during adolescence—the most dramatic shift observed across all conditions. Bioinformatics analyses further pinpointed oligodendrocytes as being robustly impacted. ELS increases H3 Ser. signal in oligodendrocyte-lineage cells, with an increase in oligodendrocyte precursor cell numbers also observed, which may impact synaptic pruning in the mPFC. Importantly, these circuit, transcriptional, and epigenetic changes were associated with impairments in cognitive flexibility during adolescence. Together, these findings reveal a novel mechanism through which ELS disrupts 5HTergic signaling in the DRN and its projections, thereby contributing to mPFC-dependent behavioral alterations associated with stress susceptibility.

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Poster

PSTR400: Early Life Stress

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR400.21/AA4

Topic: B.09. Glial Mechanisms

Support: Lafayette Parish Medical Society Endowed Professorship
UL Lafayette, GSO
McNair Scholars Program
University of Louisiana at Lafayette Undergraduate Mini Grant
University of Louisiana at Lafayette Sustainability Award

Title: Increased depressive behavior and reactive microglia in a mouse model of early life adversity

Authors: T. SUPTY[1], T. L. CAIN[3], A. DOBARD[2], ***K. M. SMITH**[1];
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Abstract: Early life is a crucial window for neurodevelopment which is greatly shaped by environmental influences. Adverse Childhood Events (ACEs), encompassing a range of psychosocial stressors experienced before the age of 18, are strongly associated with increased risk for developmental difficulties, neuropsychiatric disorders, mood disorders, and addiction. To investigate the neurodevelopmental impacts of early life stress, we utilized maternal separation (MS) model in tgFGFR1-eGFP and Swiss Webster mice of both sexes. Maternal separation is a widely used experimental paradigm to study early life stress in rodents that affects the development of the Hypothalamic-Pituitary-Adrenal (HPA) axis and the methylation status of the glucocorticoid receptor (GR) promoter, crucial factors in emotional processing and anxiety-related behaviors. We separated mouse pups from their dam for 3 hours daily from postnatal day 1 to 14. Since neuroinflammation is increasingly recognized as a key contributor to the pathology and progression of various neuropsychiatric disorders, we focused on exploring changes in microglia population as well as expression of Interleukin-1β and complement protein C1q via qPCR following maternal separation. The microglia count and morphology were assessed by immunohistochemistry using microglia marker protein, ionized calcium binding adaptor molecule 1 (Iba1). We also examined the phagocytic activity of microglia using immunohistochemistry for MER proto-oncogene tyrosine kinase (MerTK). We found a significant increase in microglia number and more ameboid, activated microglial morphology in the anterior cingulate cortex (ACC) in stressed mice. Imbalances in active-state and resting-state microglia disrupt normal synaptic growth, pruning and function and can potentially lead to reduced resiliency to future stressors. However, no significant change was observed in MerTK expression suggesting that the shift toward activated morphology may not be associated with phagocytic pathway upregulation. Furthermore, behavioral assessment in adulthood revealed that mice subjected to maternal separation show increased immobility time in the tail suspension test, reflecting behavioral despair and a depressive-like phenotype. The findings integrate immediate changes in neuroinflammation profile as well as long-term neurobehavioral consequences of early life stress events like maternal separation. This is crucial to understand the pathophysiological link between ACEs and increased risk of neuropsychiatric disorders,

potentially paving the way for development of targeted interventions to mitigate long-term effects.

Disclosures: T. Supty: None. **T.L. Cain:** None. **A. Dobard:** None. **K.M. Smith:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.01/AA5

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant RO1DA049737

Title: Investigating the effect of dual use of smoking and vaping products on blood-brain barrier using in-vitro and in-vivo models

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Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX

Abstract: Background Tobacco product use remains the leading cause of preventable disease and death in the US, with approximately 49.2 million people using some form of tobacco product. Many individuals continue to smoke while vaping, representing a type of dual-use. Over half of dual users have reported using vapes to quit or cut back on cigarette smoking. Vapes are often perceived as a less harmful alternative to smoking. However, the limited data and potential impact of dual-use on the blood-brain barrier (BBB) remain poorly understood. So, this study aims to investigate the effect of dual exposure to smoking and vaping on the BBB. **Methods** In an *in-vitro* setup brain endothelial cells (bEnd3) and primary astrocytes from mouse source were exposed to three dual combinations with combustible cigarettes (smoking): electronic cigarettes (vaping) ratios of 1:1, 3:1, and 1:3 along with combustible (C-cig) and electronic cigarettes (E-cig) alone, for both short 24 hours and prolonged 5 days duration. Assessment includes cell viability (MTS assay), barrier permeability with 3 different models: bEnd3 monolayer, bEnd3 using astrocyte-conditioned media (ACM), and co-culture of bEnd3 and astrocytes, along with testing sodium fluorescein assay (NaF) permeability. Western blot was carried out targeting oxidative stress markers, and tight junction (TJ) protein expression. In an *in-vivo* model, male C57BL/6 mice were exposed to similar experimental conditions for 7 days. Assessment includes % body weight change and checking TJ protein expression using brain homogenates by Western blot analysis. **Results** Increased NaF permeability was observed in all dual groups, including C-cig and E-cig, in a monolayer setup. However, in the ACM and co-culture setups, increased permeability was noted in C-cig and the 1:1 dual group after a brief 24-hour exposure. A decrease in ZO-1 expression after 24-hour exposure and claudin-5 expression after 5 days was observed in the 1:1 dual group, suggesting progressive tight junction disruption. Furthermore, elevated levels of antioxidative markers, such as NQO1 and HO-1, were detected in both bEnd3 and astrocytes across all dual groups, indicating a shift in oxidative stress. With our *in-vivo*

studies, we observed a significant change in % body weight in the C-cig, 1:1, and 3:1 dual group (n=5). Decreased ZO-1 expression in the 1:1 dual group was observed, suggesting compromised barrier integrity. **Conclusion:** Dual use of smoking and vaping is often misinterpreted as a safer alternative due to the perceived reduction in C-cig exposure. However, our preliminary *in-vitro* and *in-vivo* data suggest that the combinations may not provide a safer alternative.

Disclosures: S. Jadhav: None. **K. Akter:** None. **D. Mara:** None. **T.J. Abbruscato:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.02/AA6

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: DFG LI 858/12-1 Projectnumber: 505539112

Title: The brain is protected from the effect of inflammation in different colitis mouse models

Authors: *E. ROBERT[1], M. GONZALEZ ACERA[2], J. VON WITTGENSTEIN[1], J. PATANKAR[2], D. C. LIE[1];
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Abstract: Patients suffering from inflammatory bowel diseases (IBD), e.g. Crohn's disease or ulcerative colitis, frequently have comorbidities like anxiety and depression, which suggests potential effects of IBD on the brain. Yet, the pathogenic mechanisms by which gut inflammation could affect the brain are unknown. Recent studies on preclinical mouse models have shown that neuronal activity is increased in the amygdala and the insular cortex of mice suffering from Dextran-Sodium-Sulfate-induced colitis. Some brain regions of DSS-treated mice displayed an inflammatory response indicated by an elevated expression of glial fibrillary acidic protein (GFAP) mRNA in the hippocampus and Cyclooxygenase-2 (COX-2) mRNA in the hippocampus and hypothalamus. Therefore, we hypothesized that different brain regions have specific sensitivities to colitis. To gain deeper insight into the effects of colitis, we analyzed brain region-specific transcriptomes and cell based inflammatory signatures of hippocampus, amygdala, hypothalamus and insular cortex in several colitis mouse models - genetically induced, chemically induced and T-cell driven. Immunohistochemical analyses of astrocytes and microglia showed no significant changes between the brains of control and treated/KO mice in all three colitis models. Also, the number of differentially expressed genes was limited and presented no signature of a brain-region specific inflammatory response. However, the mRNA of lipocalin-2 (*Lcn2*) was consistently upregulated in at least one brain region in all three colitis models. LCN2 is a secreted protein known to be expressed by astrocytes and endothelial cells in the brain during peripheral inflammation but also brain injury. Previous studies showed that LCN2 can alleviate inflammatory processes in the gut, liver, lungs and the brain and might also

influence the permeability of the blood brain barrier (BBB). Our preliminary data shows elevated LCN2 expression in specific brain regions of colitis induced mouse models in CD31+ cells. CD31 is a marker for endothelial cells and a key component of the BBB. Therefore, we hypothesize that LCN2 could potentially trigger protective mechanisms for the brain in the context of colitis. Taken together, colitis did not trigger an inflammatory response in hippocampus, amygdala, hypothalamus and insular cortex. Interestingly, the secreted protein LCN2 emerged as a candidate which modulates the brain's response to peripheral inflammation in this context. In the future, we plan to investigate whether LCN2 can exert such putative immune-modulatory effect in the brain and its underlying mechanisms.

Disclosures: E. Robert: A. Employment/Salary (full or part-time): Friedrich-Alexander Universität Erlangen-Nürnberg. **M. Gonzalez Acera:** None. **J. Von Wittgenstein:** None. **J. Patankar:** None. **D.C. Lie:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.03/AA7

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NS135147
NS141403

Title: The impact of bacterial pneumonia on cerebral blood flow and neurovascular unit integrity over time

Authors: *S. D. CHANEY, A. R. NELSON;
Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL

Abstract: Background: Many survivors of severe bacterial pneumonia, and other severe lung injury, suffer from incident dementia. Patients who have had pneumonia and other infections are at a higher risk for developing Alzheimer's disease and related dementias (ADRD). Previously, we found that bacterial pneumonia causes blood-brain barrier breakdown, astrocyte reactivity, and tau hyperphosphorylation 24 hours post-infection. Here, we investigated whether bacterial pneumonia first causes blood-brain barrier breakdown or gliosis in the brain. Method: PrismPlus mice were intratracheally infected with Pseudomonas aeruginosa (PA103, ExoU and ExoT competent) or saline for control. The mice were imaged longitudinally over several days through a cranial window. Cerebral blood flow was measured by laser speckle imaging. Labeled dextran was injected retro-orbitally to measure blood-brain barrier integrity. Microglia, astrocytes, neurons, oligodendrocytes, and dextran were imaged by multiphoton microscopy. Result: Differences in global cerebral blood flow were measured over several days following infection. Additionally, integrity of the neurovascular unit was determined by multiphoton imaging of the PrismPlus mice. Conclusion: Bacterial pneumonia negatively impacts the neurovascular unit,

including the blood-brain barrier breakdown, glia, and neurons. Understanding how pneumonia causes these events and how this can lead to increased risk of ADRD is extremely timely and important. Future studies should focus on characterizing pneumonia-induced neurovascular unit dysfunction in patients.

Disclosures: S.D. Chaney: None. **A.R. Nelson:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.04/AA8

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant R01DA054921

Title: Sars-cov-2 spike protein impairs blood-brain permeability via sigma-1r activation in brain microvascular endothelial cells

Authors: E. BRAILOIU[1], P. ZHAO[1], H. WITTORF[2], ***G. C. BRAILOIU**[3];
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Abstract: SARS-CoV-2, similarly to other coronaviruses, can enter the brain via hematogenous or neural propagation and can produce blood-brain barrier (BBB) impairment. Using the rat brain microvascular endothelial cells (RBMVEC) that express ACE2, the target for SARS-CoV-2 Spike protein, as a model for BBB, we examined the effect of the SARS-CoV-2 Spike protein (active trimer, full length, Acrobiosystems Inc., Newark, DE) on the cytosolic calcium (Ca^{2+}), mitochondrial Ca^{2+} and mitochondrial reactive oxygen species (ROS) and the role of Sigma-1R , an intracellular receptor located on the endoplasmic reticulum. We performed binding studies in Sigma-1R stable transfected HEK293 cells, using the Sigma-1R ligand ^{3}H-pentazocine and determined that Spike protein produced a dose-dependent displacement of ^{3}H-pentazocine indicating specific binding to Sigma-1R. SARS-CoV-2 Spike protein produced a concentration-dependent increase in the cytosolic Ca^{2+} concentration (measured with Fura-2 AM methods), mitochondrial Ca^{2+} (measured in cells loaded with Rhod-2-AM) and mitochondrial ROS (measured with MitoSOX Red). The effect of Spike protein on cytosolic Ca^{2+}, mitochondrial Ca^{2+} and mitochondrial ROS was reduced by Sigma-1R antagonist, BD-1047. These results support a critical role for Sigma-1R in the increase in cytosolic Ca^{2+}, mitochondrial Ca^{2+} and mitochondrial ROS produced by SARS-CoV-2 Spike protein.

Disclosures: E. Brailoiu: None. **P. Zhao:** None. **H. Wittorf:** None. **G.C. Brailoiu:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.05/AA9

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Finnish Pharmacological Society
The Finnish Medical Foundation
Sigrid Jusélius Foundation
Emil Aaltonen Foundation
iCANDOC Doctoral Education Pilot in Precision Cancer Medicine

Title: Peripheral alpha-2 antagonism modulates the effects of dexmedetomidine on the glymphatic flow independently of slow-wave activity

Authors: *S. C. JALONEN[1,2,3], H. ANTILA[4,5], N. PERSSON[2,3], T. J. LOHELA[7,3], M. PELTONIEMI[1,2,3], T. O. LILIUS[1,2,3,6];
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Abstract: Dexmedetomidine, an α2-adrenergic agonist, enhances the cerebrospinal fluid (CSF)-mediated waste-clearing glymphatic system, likely via reduced central noradrenergic tone and increased slow-wave activity (SWA). In parallel, α2-agonists induce a plethora of systemic effects such as peripheral vasoconstriction, plasma hyperosmolality, and blood pressure alterations, all possibly influencing CSF dynamics. Using a peripherally selective α2-antagonist, we investigated the significance of peripheral effects on the glymphatic-enhancing properties of dexmedetomidine. First, the spectral signature was assessed with EEG alongside the assessment of peripheral parameters. Next, the effects on blood pressure and blood flow were measured. Finally, CSF flow was assessed by quantifying the whole-body distribution of an intracisternally infused radiolabeled tracer, [99mTc]Technetium-diethylenetriaminepentaacetic acid ([^{99mTc}]Tc-DTPA), with single-photon emission computed tomography/computed tomography (SPECT/CT). Peripheral antagonism had no influence on the dexmedetomidine-induced SWA, while simultaneously ameliorating hyperglycemia, hyperosmolality, and peripheral vasoconstriction. Moreover, peripheral antagonism increased the concentration of [^{99mTc}]Tc-DTPA in the intracranial space and cortical availability, while decreasing the availability in the spinal canal. Outside the CNS, the availability and excretion of [^{99mTc}]Tc-DTPA were elevated. In conclusion, co-administration of a peripheral α2-antagonist facilitated CSF influx into the intracranial space and cortical region during dexmedetomidine anesthesia, suggesting that peripheral modulation of α2-receptor activity may create an optimized environment for glymphatic influx independent of central SWA.

Disclosures: S.C. Jalonen: None. **H. Antila:** None. **N. Persson:** None. **T.J. Lohela:** None. **M. Peltoniemi:** None. **T.O. Lilius:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.06/AA10

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Characterization of a human blood brain barrier triculture system consisting of endothelial cells, astrocytes, and pericytes on a high electrode density semiconductor.

Authors: *J. LAWSON, C. PERRY, A. JOHNSON, H. RUETH, K. REMONDINI, M. L. HENDRICKSON, K.-D. CHOI;
BrainXell, Madison, WI

Abstract: The blood brain barrier is a highly selective membrane that separates the brain from peripheral blood flow and is made up of brain microvascular endothelial cells (BMECs), which are connected by tight junctions and form the luminal boundary. On the abluminal side of the BMECs, are pericytes (PC), astrocytes (AC), and neurons along the basal lamina; all together they make up the brain's neurovascular BBB. When disruption occurs, acute pathological inflammatory cascades can arise and when left unchecked can lead to BBB breakdown and chronic neurodegeneration. Alzheimer's Disease (AD), Parkinson's Disease (PD), and Multiple Sclerosis (MS) have been linked to neuroinflammation and there is a rising need for new therapeutic strategies and research models. Many neurodegenerative diseases do not have adequate treatments which is due to the BBB's highly selective nature, which allows only a small percentage (> 2%) of drugs to pass. Although there has been progress in defining blood brain barrier properties, the molecular mechanisms of barrier function still need to be elucidated. Consequently, there is an increasing need for new preclinical models that more accurately mirror human physiology and response to therapeutic compounds. Human iPSC-derived Organ-on-a-chip systems are emerging as a promising technology that can better mimic in vivo physiological and pathological function than primary animal tissue without the use of costly animals. Here we've demonstrated that our human iPSC-derived triculture BBB model, on a cutting-edge semiconductor-to-live-cell-interface, can be modulated with compounds and conditioned culture medium. Quantitative-PCR shows changes in abundance levels of tight junction and transporter transcripts in response to culture conditions and immunocytochemistry shows an increase in VE-cadherin fluorescence in co- and tri-cultures when compared to monocultures. Our human BBB-on-a-chip offers a physiologically relevant model that can be used for functional barrier, dynamic cell-cell signaling, and cell migration studies which could advance our understanding of neurological diseases and lead to therapeutic drug discoveries.

Disclosures: J. Lawson: A. Employment/Salary (full or part-time): BrainXell. **C. Perry:** A. Employment/Salary (full or part-time): BrainXell. **A. Johnson:** A. Employment/Salary (full or part-time): BrainXell. **H. Rueth:** A. Employment/Salary (full or part-time): BrainXell. **K. Remondini:** A. Employment/Salary (full or part-time): BrainXell. **M.L. Hendrickson:** A.

Employment/Salary (full or part-time): BrainXell. **K. Choi:** A. Employment/Salary (full or part-time): BrainXell.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.07/AA11

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: The effect of gut-derived metabolites on the blood-brain barrier

Authors: *A. NUSBAUM[1], S. CANFIELD[2], B. NEEDHAM[1];
[1]IU Sch. of Med., Indianapolis, IN; [2]IU Sch. of Med., Terre Haute, IN

Abstract: The blood-brain barrier (BBB) serves as a selective semi-permeable membrane at the junction of the peripheral and central nervous systems. One intriguing way the BBB can be influenced is through gut-derived metabolites—molecules produced by the community of bacteria in the intestinal tract. These metabolites can travel through the circulatory system, affecting BBB integrity and potentially altering brain function. As research into the potential effects of circulating gut-derived metabolites on BBB integrity expands, exploring those that remain uninvestigated is crucial. Notable molecules such as imidazole propionate (ImP), commendamide, and 3-hydroxykynurenine (3-HK) have unclear effects on the BBB. Therefore, this study aimed to examine the impact of ImP, commendamide, and 3-HK on the BBB, with two main objectives: characterizing the disruption of tight junctions and assessing barrier functional integrity. Our work utilized an *in vitro* model of human induced pluripotent stem cells differentiated into brain microvascular endothelial cells (BMECs) to evaluate critical barrier properties. This *in vitro* model closely mimics *in vivo* conditions and allows us to measure changes in tight junction localization through immunostaining and quantification. Significant alterations in claudin-5 and occludin localization occurred after the application of ImP, while measurable disruptions in the localization of occludin were noted after treatment with 3-HK. Observable changes in claudin-5 localization were detected following commendamide treatment. Additionally, we measured electrical resistance across the BMEC monolayer using transendothelial electrical resistance (TEER), indicating changes in barrier integrity. Alongside TEER, we conducted sodium fluorescein permeability assays. Both methods evaluated the effects of these gut-derived metabolites on barrier integrity and permeability, suggesting that circulating gut-derived metabolites could influence the functional integrity of the barrier. Enhancing our understanding of gut-derived metabolites that modulate BBB integrity, such as ImP, commendamide, and 3-HK, can help redirect research from mere associations to more mechanistic investigations. Furthermore, this research heightens our understanding of the gut-brain axis, suggesting that the BBB is a key point for gut-derived metabolites to exert harmful effects.

Disclosures: A. Nusbaum: None. **S. Canfield:** None. **B. Needham:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.08/AA12

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NINDS RF1 NS135617

Title: Heterogeneity of CNS Vasculature and the Blood-Brain Barrier

Authors: *S. XIA[1], Z. ZHAO[2];
[2]Physiol. and Neurosci., [1]USC, Los Angeles, CA

Abstract: The blood-brain barrier (BBB) plays key roles in maintaining brain homeostasis and functions. However, it is not a uniform structure but exhibits regional and cellular heterogeneity that reflects and supports the functional complexity of the central nervous system. This heterogeneity influences the permeability, transport mechanisms, and immune surveillance across different brain regions, shaping how various areas develop and mature, or respond to injuries and diseases. Understanding BBB heterogeneity is also crucial for developing targeted therapies, as it directly impacts drug delivery and the progression of neurological diseases such as Alzheimer's, multiple sclerosis, and stroke. Using single cell transcriptomics, we identified new BBB specific vascular markers and transporters. We further confirmed their specificity using new transgenic knock-in reporter and Cre driver models. These new genetic tools and BBB transporter map will help us further explore the vascular heterogeneity and provide a better understanding of the BBB biology and specialization.

Disclosures: S. Xia: None. **Z. Zhao:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.09/AA13

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Breaking Molecular Barriers: A Multiomic Atlas of the Human Choroid Plexus

Authors: *N. KALIA[1], C. WIJESINGHEGE[2], J. JAUREGUI-LOZANO[2], C. CHENG[2];
[1]Univ. of California, San Diego, La Jolla, CA 92093, USA, La Jolla, CA; [2]UCSD, La Jolla, CA

Abstract: Border tissues such as the choroid plexus (ChP) serve not only as physical barriers but also as critical neuro-immune interfaces. Despite their essential role in immune surveillance, they remain understudied, particularly in neurodegeneration. While single-cell transcriptomic studies have revealed the transcriptional landscape of ChP, its epigenetic complexity remains largely unexplored. Here, we present the first comprehensive multiomic single-nucleus atlas of postmortem human ChP. Using single-nucleus RNA sequencing (snRNA-seq) and ATAC sequencing (snATAC-seq) on 16 high-quality human samples via the 10x Genomics Multiome platform, we simultaneously profiled gene expression and chromatin accessibility. After quality filtering, we retained 28,405 nuclei with paired transcriptomic and epigenomic profiles. Cell type annotations derived independently and jointly from both modalities based on known marker genes, were consistent and robust. We identified major ChP populations, including epithelial, mesenchymal, immune, ependymal, and endothelial cells, as well as border-associated macrophages (BAMs) and T cells. To define regulatory programs, we constructed transcription factor (TF)-centered gene regulatory networks using Pando, which integrates chromatin accessibility and gene expression by linking TF motifs to correlated target gene expression. This enabled identification of cell-type-specific regulatory circuits. Epithelial cells showed enrichment for LYL1, IRF8, FOXD2, CEBPA, and POU2F2 networks, suggesting roles in vascular signaling, immune crosstalk, barrier integrity, and metabolism. Ependymal cells were enriched for CREB5, TSHZ2, RFX4, NFIB, and RFX8, associated with ciliogenesis and differentiation. Mesenchymal cells featured TFs ZNF883, ESRRG, and HDX, linked to matrix remodeling and metabolism. BAMs showed networks driven by ZNF618, ETV6, and PRDM16, reflecting macrophage identity and polarization. To further investigate regulatory dynamics, we constructed TF-target networks and found most genes were co-regulated by multiple TFs, suggesting cooperative control to maintain the robustness of the system. This multiomic ChP atlas provides novel insight into gene regulation across barrier-resident cell types and serves as a valuable resource for studying ChP involvement in neurodegenerative disease.

Disclosures: **N. Kalia:** Other; Contributed Equally. **C. Wijesinghege:** Other; Contributed Equally. **J. Jauregui-Lozano:** None. **C. Cheng:** Other; Senior Author.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.10/AA14

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Choroid plexus brain barrier gates acetaldehyde entry into the brain

Authors: ***J. XU**[1], S. WEI[2], Y. LIN[3], D. M. LOVINGER[4], L. ZHANG[5];
[1]Lab. of NeurogeneticsNIAAA, NIH, Rockville, MD; [2]LIN, NIAAA/NIH, Rockville, MD; [3]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; [4]Chief, Lab. Integrative Neurosci, Natl. Inst. on Alcohol Abuse and Alcoholism, Olney, MD; [5]NIAAA, NIH, Rockville, MD

Abstract: Ethanol (EtOH) is quickly metabolized into acetaldehyde (AcH) and acetate in the human body. AcH contributes to alcohol's psychoactive effects and toxicity. However, the mechanisms by which circulating AcH enters the brain remain unclear. The brain is protected from exposure to circulating harmful chemicals by two major barriers: the blood-CSF barrier (BCSFB) and the blood-brain barrier (BBB). ChP epithelial cells produce a large portion of cerebrospinal fluid (CSF) and act as the sites of blood-CSF barrier (BCSFB) in the brain ventricles. Emerging evidence suggests that the ChP serves as a detoxification center, removing harmful compounds from blood circulation or brain metabolism. Here, we examined the levels of EtOH and the metabolites, AcH and acetate, in the cerebrospinal fluid (CSF) of the lateral ventricle, where choroid plexus (ChP) is located, in freely moving mice following a systemic administration of either EtOH or AcH. The concentration of CSF AcH, but not EtOH or acetate, was significantly higher than AcH levels detected in interstitial spinal fluid (ISF) of various brain subregions. The selective entry of CSF AcH across ChP-brain barrier (ChPBB) was also evident in $ALDH_2$ deficient mice, which lack the enzyme required to break down AcH. This barrier was particularly vulnerable to chronic EtOH exposure, as CSF levels of albumin (Alb) and AcH were 3-4 times higher than those in ISF. A similar scenario was also observed in aged mice, whose compromised ChPBB was permeable to CSF Alb and AcH. In both chronically EtOH-exposed and aged mice, elevated CSF AcH levels were positively correlated with CSF Alb concentrations. Notably, the increases in both Alb and AcH were detected only when using microdialysis probes with large, but not small, molecular weight cutoffs, suggesting a carrier-mediated mechanism for AcH transport. Both chronic EtOH exposed mice and aged mice consumed significantly less EtOH, consistent with our observation that lateral ventricular microperfusion of AcH via mini pumps suppressed alcohol consumption and preference. Thus, the ChPBB serves as a key route for peripheral AcH entry into the brain, especially during chronic alcohol consumption and aging. This barrier plays a critical role in alcohol consumption, intoxication and toxication.

Disclosures: J. Xu: None. **S. Wei:** None. **Y. Lin:** None. **D.M. Lovinger:** None. **L. Zhang:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

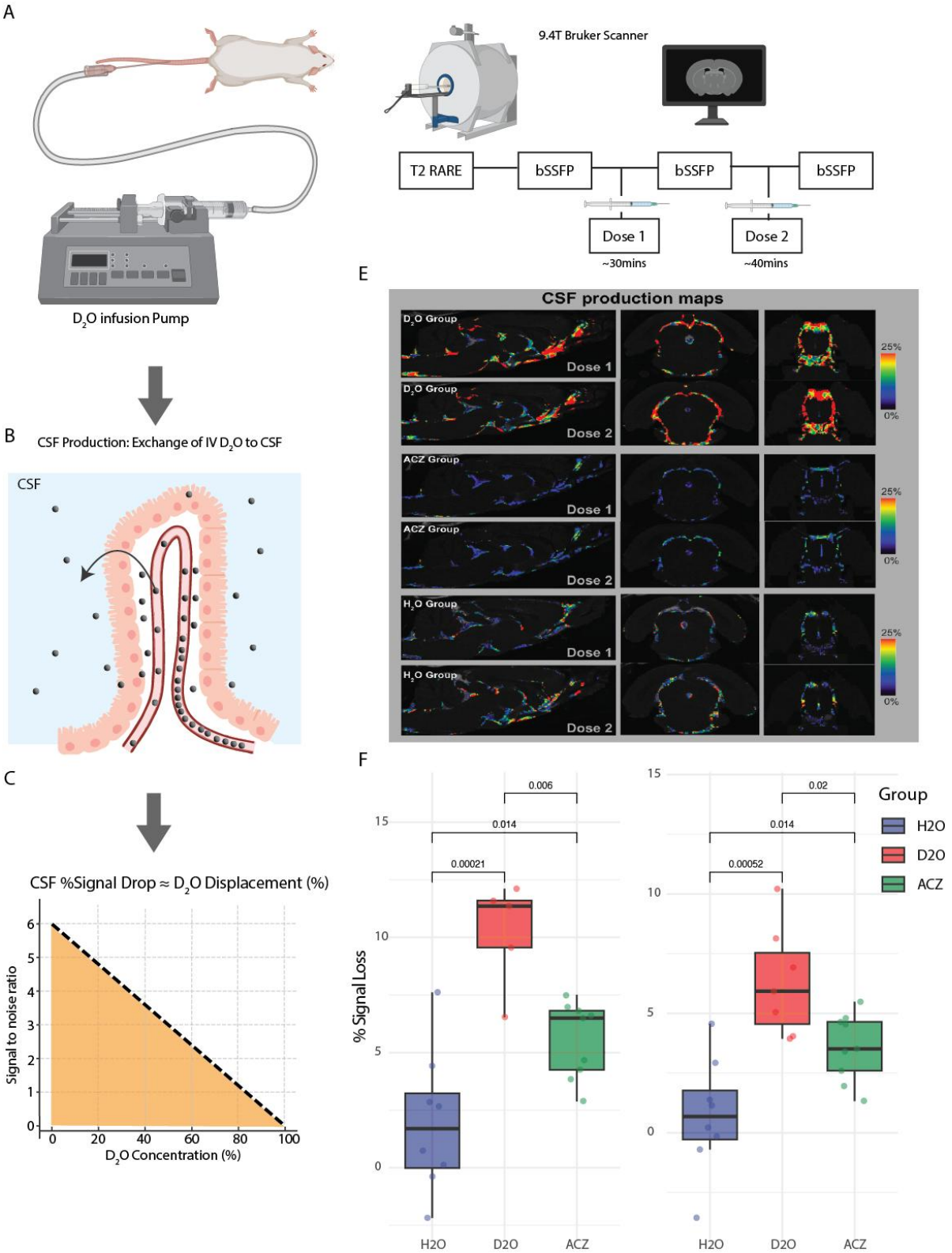
Program #/Poster #: PSTR401.11/AA15

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Indirect Deuterium Exchange Imaging for Non-Invasive High-Resolution CSF Production Mapping

Authors: *D. DE[1], **A. NAZERI**[3], H. HOSSEINI[1], N. BATEMAN[1], S. PAN[1], J. QUIRK[1], J. STRAHLE[2];
[1]Washington Univ. in St. Louis, Sch. of Med., Saint Louis, MO; [2]Washington Univ. in St. Louis, Sch. of Med., St. Louis, MO; [3]Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Motivation: Cerebrospinal fluid (CSF) is crucial for brain homeostasis and waste clearance; its disruption impairs brain fluid dynamics, contributing to neurological disease. Existing non-invasive CSF MRI methods often use indirect proxies, miss cumulative production, and have limited spatial resolution/signal-to-noise ratio. We used deuterium oxide (D_2O) as an IV negative contrast agent and water analog, where 1H-MRI signal loss is proportional to its displacement of new CSF water.
Methods: Twenty-four male Sprague Dawley rats (4-7 mo) were grouped: D2O (n=7); Acetazolamide (ACZ)/D_2O (n=9); H2O controls (n=8). The ACZ/D_2O group received IP ACZ (40 mg/kg) pre-D_2O infusion to suppress CSF production. Controls received an equivalent IV dose of H_2O saline. High-resolution 3D balanced steady-state free precession (b-SSFP; 110 μm^3 voxel size) was acquired on a 9.4T Bruker MRI before and after D_2O infusion to detect deuterium-related CSF signal changes. Global % signal reduction from D_2O displacement was computed from pre/post-infusion images in segmented CSF. Region-of-interest (ROI) analysis used a custom CSF atlas.
Results: IV D_2O induced marked signal attenuation within the CSF compared to control rats receiving H_2O (first dose: 10.0% [SD: 2.3%] vs. 2.0% [SD: 3.1%], $p = 2.1 \times 10^{-4}$; second dose: 6.3% [SD: 2.3%] vs. 0.7% [SD: 2.4%], $p = 5.2 \times 10^{-4}$). Rats pretreated with ACZ demonstrated intermediate signal loss following D_2O infusion (first dose: 5.6% [SD: 1.7%]; second dose: 3.6% [SD: 1.4%]), consistent with partial inhibition of CSF production. Follow-up ROI analysis across 13 ventricular and cisternal compartments revealed a similar pattern of group-wise signal attenuation, with the ACZ group exhibiting intermediate reductions between the D_2O and H_2O groups.
Conclusion: These findings establish deuterium displacement exchange imaging as a feasible high-resolution, non-invasive method for assessing CSF production and its pharmacological modulation. Future work will refine quantification and explore applications in various brain disorders.



Disclosures: D. De: None. **A. Nazeri:** None. **H. Hosseini:** None. **N. Bateman:** None. **S. Pan:** None. **J. Quirk:** None. **J. strahle:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.12/AA16

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Changed functional protein expression in human choroid plexus epithelial cells containing Biondi body amyloid inclusions

Authors: *B. A. JOHNSON[1], F. GARZA[2], M. NEEL[3], E. S. MONUKI[4];
[1]Pathology & Lab. Med., Univ. California, Irvine, Irvine, CA; [2]Univ. of California, Irvine, Irvine, CA; [3]UC Irvine, Whittier, CA, ; [4]UC Irvine Sch. of Med., Irvine, CA

Abstract: Through their production and conditioning of cerebrospinal fluid (CSF), choroid plexus epithelial cells (CPECs) provide important homeostatic support for the brain and spinal cord. Age-related changes to CPECs likely contribute to declines in CSF production and/or barrier integrity, thereby putting the CNS at risk for neurodegenerative disease. However, there have been no data on whether these age-related changes are pathological (i.e., whether they impair function). Biondi body amyloid inclusions accumulate abruptly in CPECs in midlife (around 50-65 years), affecting most CPECS in most people over seventy. To determine if Biondi body accumulation might impact CPEC function, we stained slices of postmortem choroid plexus from eight individuals using the fluorescent amyloid dye thioflavin S along with antibodies against a variety of proteins important to CPEC function (the secretory protein transthyretin, the intracellular ionic regulators carbonic anhydrase 2 and anion exchange protein 2, the water channels aquaporin and NKCC1, and the tight junction protein claudin 5), and we compared staining intensities in CPECs with and without Biondi body inclusions. We found significant effects (increases and decreases) involving all antigens tested, suggesting that Biondi bodies likely do affect CPEC function, but there were striking differences in the patterns and the degrees of the effects among the individuals. These individual differences may be associated with different Biondi body morphologies and/or spatial distributions, and they may also indicate differences in Biondi body composition and/or different stages of the cellular response to the presence of Biondi bodies.

Disclosures: B.A. Johnson: None. **F. Garza:** None. **M. Neel:** None. **E.S. Monuki:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.13/BB1

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH T32 Grant AG000096
NIH T32 Grant AG073088
NIH P30 Grant AG066519

Title: Manual and deep learning analysis of Biondi bodies in human choroid plexus reveal distinct morphologies, spatial clustering, and possible prion-like spread

Authors: *M. J. NEEL, B. A. JOHNSON, E. S. MONUKI;
UC Irvine Sch. of Med., Irvine, CA

Abstract: The choroid plexus (ChP) plays a key role in brain homeostasis and its dysfunction may play a role in brain aging and diseases such as Alzheimer's disease (AD). However, many age and disease related changes within the ChP are understudied. One of these changes is the formation of Biondi bodies (BB), amyloid aggregates unique to the ChP. In this study, we characterized the morphology, prevalence, spatial distribution, and load of BBs using both manual and deep-learning (DL) quantification approaches. To study BB morphology, we used confocal imaging of whole-mount, thioflavin-S stained ChP autopsy samples across 37 individuals to visualize BBs in three dimensions for blinded counting and categorization into distinct morphologies. For BB prevalence, spatial distribution, and load, we developed a rigorous manual counting methodology to measure BB prevalence from thioflavin-S stained whole slide images across 77 individuals. This data was used to train DL models based on ResNet, RetinaNet, and U-Net architectures. These models were subsequently used to analyze the spatial distribution and load of BBs. Our findings revealed that BBs exhibit distinct and circumscribed shapes, with inter-individual variability in the relative distributions of these morphologies. Notably, these relative distributions were marginally associated with AD ($p=0.055$), where individuals with AD exhibited a higher abundance of more complex BB morphologies. Analysis of BB prevalence demonstrated a sigmoidal increase with age, which was slower in females, but did not find an association with AD. We found that BB prevalence correlated with the relative proportion of certain morphologies, suggesting pathways of BB maturation. Additionally, we identified interesting outliers in our study: two cases involving diabetes (positive outliers) and one individual with the AD-protective APOE $\varepsilon2/\varepsilon2$ genotype (negative outlier). The DL models trained on our manual data performed well and recapitulated the key findings of our manual analysis. Furthermore, using these DL models, we observed significant spatial clustering of BBs in every ChP examined, suggesting possible prion-like spread. Altogether, these results show that BBs increasingly impact the ChP with age and highlight DL models as a valuable tool for automating data collection and enabling new studies.

Disclosures: M.J. Neel: None. **B.A. Johnson:** None. **E.S. Monuki:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.14/BB2

Topic: G.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH T32 Grant AG073088-04
Pilot award from the UCI Center for Neurotherapeutics

Title: Vascularized choroid plexus epithelial organoids from human iPSCs for drug delivery via the cerebrospinal fluid pathway

Authors: *V. ESPERICUETA, M. ANDRADE, I. SANCHEZ, Q. SMITH, E. S. MONUKI; Univ. of California, Irvine, Irvine, CA

Abstract: A major roadblock in therapeutic intervention for neurological and neurocognitive disorders is the inability of conventional drugs to penetrate the blood-brain barrier (BBB). An alternative route into the CNS is through the blood-cerebrospinal fluid (CSF) barrier. Our project aims to develop an innovative, patient-specific, drug delivery system based on vascularized choroid plexus epithelial cell (CPEC) organoids derived from human induced pluripotent stem cells (iPSCs). CSF-producing CPEC organoids could transform CNS therapeutics as delivery vehicles that bypass the BBB and vascularization can help facilitate integration into recipient and provide long-term viability of the organoids. Based on a simplified CPEC derivation protocol for monolayer cultures (Masters et al., *Nat Commun* 2025), we developed a CPEC organoid model using only bone morphogenic protein 4 (BMP4) as the driver for CPEC differentiation. Immunostaining with the well-established CPEC marker transthyretin (TTR), the cilia marker ARL13B, and the basal membrane transport protein AE2 revealed the apical surface was outward (media) facing, while the basal compartment faced inward. Much like the actual tissue, the CPECs formed papillary-like structures on the outer edges of the organoid. To vascularize CPEC organoids, we co-cultured vascular organoids, composed of endothelial cell networks and pericytes, with CPEC organoids in U-bottom wells. CPEC organoids expressing green fluorescent protein (GFP) were successfully fused with non-GFP vascular organoids. Fused organoids significantly increased secretion of TTR compared to CPEC organoids alone suggesting enhanced CPEC secretory function. Further characterization of the vascularized CPEC organoids is ongoing to understand the extent of functional enhancement and rationale for use of these assembloids as neurotherapeutic delivery systems.

Disclosures: V. Espericueta: None. **M. Andrade:** None. **I. Sanchez:** None. **Q. Smith:** None. **E.S. Monuki:** None.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.15/BB3

Topic: D.03. Stroke

Support: JSPS KAKENHI Grant Number 24K23435
JSPS KAKENHI Grant Number 21H05049

Title: The contribution of CSF dynamics to cerebral edema formation after ischemic stroke

Authors: *T. FURUMIYA, T. ITOKAZU, T. YAMASHITA;
The Univ. of Osaka, Suita, Osaka, Japan

Abstract: Ischemic stroke occurs when a cerebral vessel is obstructed by a clot. Many patients suffer from disabilities even after the successful reperfusion treatments such as thrombolysis or thrombectomy. Cerebral edema is among secondary brain injuries, which is a therapeutic target after ischemic stroke. We are interested in the contribution of cerebrospinal fluid (CSF) dynamics to cerebral edema formation after ischemic stroke. CSF is mainly produced in the choroid plexus inside the ventricular system, reaching the subarachnoid space and then bathing the brain parenchyma as glymphatic system. After parenchymal perfusion, CSF flows into the dura where it is absorbed by dural lymphatics. A recent study suggests that CSF dynamics such as glymphatic CSF influx becomes pathological along with spreading depolarization shortly after ischemic stroke, leading to cerebral edema formation. However, the whole picture of CSF dynamics after ischemic stroke remains to be elucidated. We established middle cerebral artery occlusion (MCAO) mouse model and firstly confirmed that cerebral edema occurs within 1 hour after the MCAO onset. Interestingly, we observed that both glymphatic CSF influx to the brain parenchyma and efflux to the dural lymphatics are largely suppressed after MCAO. Basically, when cerebral edema occurs, parenchymal water in-out balance should be skewed toward the influx. Therefore, we hypothesized that CSF influx pathways other than glymphatic system might be activated after MCAO. Here we focused on transependymal CSF influx, which is a minor CSF flow across the ependymal layer from the ventricular system to parenchyma. Surprisingly, we observed that transependymal CSF influx is potentiated shortly after the MCAO onset. In the process of investigating molecular mechanisms of increased transependymal influx, we identified dramatic morphological changes in ependymal cells induced by brain ischemia. Although the downstream molecular pathways are under investigation, we believe that this observation might bring us the general conceptual framework in CSF dynamics combining transependymal, intraventricular, glymphatic and dural lymphatic CSF flow.

Disclosures: T. Furumiya: A. Employment/Salary (full or part-time): The University of Osaka. **T. Itokazu:** A. Employment/Salary (full or part-time): The University of Osaka. **T. Yamashita:** A. Employment/Salary (full or part-time): The University of Osaka.

Poster

PSTR401: Barriers of the CNS

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR401.16/BB4

Topic: D.02. Ischemia

Support: Veterans Affairs Grant I01BX006534

Title: Targeting monocyte-derived macrophages to protect the blood-brain barrier after stroke

Authors: *C. M. DUFORT[1], X. HU[2];
[1]Neurol., Univ. of Pittsburgh Ctr. For Neurosci., Pittsburgh, PA; [2]Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In homeostasis, the brain parenchyma is protected by the blood-brain barrier (BBB), which prevents the passage of large molecules, pathogens, and leukocytes. Cellular distress unleashed by ischemia leads to BBB breakdown, which exacerbates neuroinflammation and injury. Angiogenesis is an important regenerative process after stroke, but quick generation of new vessels without mature barrier function necessitates further BBB leakage. The timing of angiogenesis must be controlled to avoid runaway inflammation. Monocytes infiltrate the brain parenchyma early after stroke, which causes edema and worsens outcomes in the short term. However, the recruitment of monocytes is essential for stroke recovery, as monocyte-derived macrophages (MDM) prevent hemorrhagic transformation, regulate angiogenesis, and promote wound repair in mouse models of cerebrovascular injury. Single-cell RNAseq analysis revealed multiple subsets of MDM in the mouse brain 3 and 14 days after transient middle cerebral artery occlusion (tMCAO), a model of ischemic stroke (n=6). At 3 days, we identified CCR2- MDM that express mRNA transcripts consistent with an anti-angiogenic, immuno-suppressive, and phagocytic phenotype. These MDM are distinctly positive for platelet factor 4 (PF4), allowing us to visualize these cells in Pf4-Cre:Ai14 (PF4-tdTomato) mice and isolate them with FACS. We transplanted PF4-tdTomato bone marrow into wild-type recipients prior to tMCAO and observed thousands of tdTomato+ MDM in the brain parenchyma 5 days after stroke that were absent in uninjured controls (n=6 tdTomato, n=2 sham). PF4+ MDM are distinct from CD206+ perivascular macrophages and we observed 58% of tdTomato-tagged MDM in close proximity to the injured vasculature. However, we also discovered a robust population of tdTomato+ MDM in the dural meninges following stroke via flow cytometry and immunostaining (n=6). Fewer than 1% of blood monocytes express tdTomato 5 days after tMCAO, leading us to examine the skull bone marrow and meninges as the predominant source of PF4+ MDM. We also selectively depleted PF4+ macrophages by crossing Pf4-Cre with Csf1r-flox mice to further understand how these MDM regulate angiogenesis and BBB integrity. We found that PF4+ MDM depletion resulted in greater endothelial cell proliferation in the infarct between days 3 and 6 of tMCAO recovery by administering BrdU. Depletion also exacerbates plasma IgG leakage into the brain parenchyma 3 days after tMCAO, suggesting worsened BBB integrity. Here, we characterize a novel population of skull bone marrow-derived MDM that appear in the brain within 72 hours of tMCAO that may be targeted to improve stroke recovery.

Disclosures: C.M. Dufort: None. **X. Hu:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.01/BB5

Topic: G.07. Biological Rhythms and Sleep

Title: Effects of Gut Microbiota Modulation on Sleep Architecture and Cognitive Function in a Chronic Sleep Disruption Model in BALB/C Male Mice

Authors: *M. NOURIZADEH[1,2], A. GHAHARI[1], P. ASLANI[3,4], M. MAJLESI[2,1], K. ALIPOUR[2], S. DAVARI[1,2], S. RASOULI[2,1], M. AMIRHOOSHANGI[1], S. MOHAMMADZADEH MOUNESYAR[2,1], M. SALIMI MOVAHHED[2], Z. SHADBAKHSH[2], M. HOSEINZADEH[1], E. KAMALI FAR[2];
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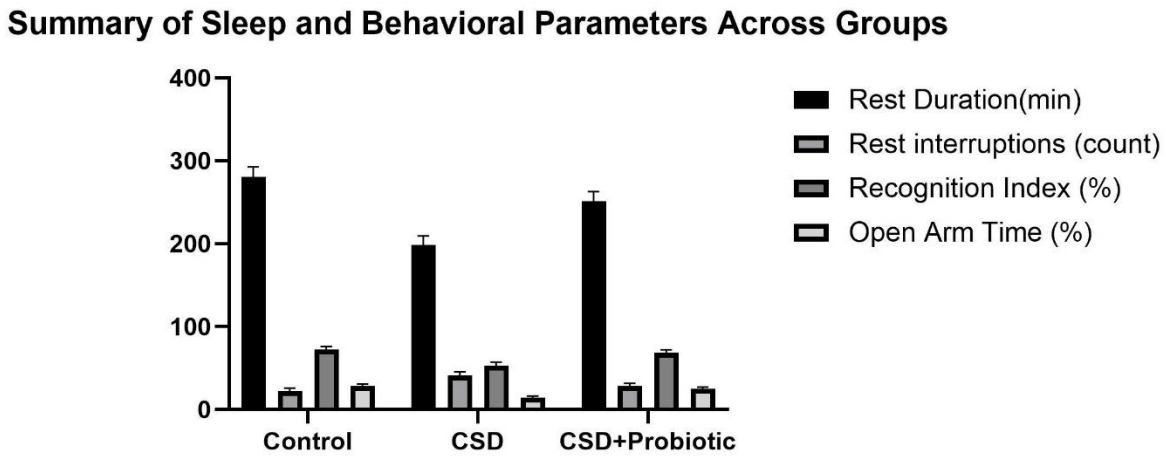
Abstract: Background: Chronic sleep disruption (CSD) impairs cognitive functions and heightens anxiety. The gut-brain axis, involving gut microbiota, is increasingly recognized as a mediator of sleep and emotional regulation. This study investigated whether gut microbiota modulation could counteract the negative behavioral effects of CSD in BALB/C mice. **Methods:** Male BALB/C mice were assigned to three groups: Control, CSD, and CSD + Probiotics. CSD was induced over 14 days via intermittent rest disturbances. The probiotic group received a commercial blend in drinking water. Behavioral tests included the Elevated Plus Maze (EPM) for anxiety and the Novel Object Recognition (NOR) test for memory. Rest duration, latency to rest, and interruption frequency were assessed through validated non-invasive observation. **Results:** CSD markedly disrupted sleep behavior and impaired cognition. Compared to controls, it reduced rest duration (198.7 ± 10.9 vs. 280.4 ± 12.3 min, $p < 0.01$), increased interruptions (41.2 ± 4.4 vs. 22.6 ± 3.1, $p < 0.01$), and prolonged latency to rest (139.6 ± 13.2 vs. 83.1 ± 10.8 sec, $p < 0.05$). NOR performance declined ($52.8 \pm 4.2\%$ vs. $72.4 \pm 3.6\%$, $p < 0.01$), while anxiety-like behavior rose in EPM ($14.3 \pm 1.9\%$ vs. $28.5 \pm 2.4\%$, $p < 0.01$). Probiotic treatment partially reversed these effects—rest duration improved (251.6 ± 11.2 min), interruptions decreased (28.4 ± 3.5, $p < 0.05$ vs. CSD), NOR scores increased ($68.1 \pm 3.9\%$), and anxiety lessened ($24.7 \pm 2.6\%$, $p < 0.05$ vs. CSD). **Conclusion:** Probiotic intervention mitigated CSD-induced behavioral deficits, underscoring the therapeutic relevance of the gut-brain axis for non-invasive management of sleep-related cognitive impairments. **Keywords:** Gut-Brain Axis, Chronic Sleep Disruption, Probiotics, Gut Microbiota, BALB/C Mice



Disclosures: M. Nourizadeh: None. **A. Ghahari:** None. **P. Aslani:** None. **M. Majlesi:** None. **K. Alipour:** None. **S. Davari:** None. **S. Rasouli:** None. **M. Amirhooshangi:** None. **S. Mohammadzadeh Mounesyar:** None. **M. Salimi Movahhed:** None. **Z. Shadbakhsh:** None. **M. Hoseinzadeh:** None. **E. Kamali Far:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.02/BB6

Topic: G.07. Biological Rhythms and Sleep

Support: R01 NS078410
NINDS-NIMH T32NS048004

Title: Sex differences in sleep responses to acute stress are related to gonadal hormones

Authors: *M. R. RALSTON, M. KAHAN, K. CHOUDHARY, E. LEE, W. KAHENDE, K. TAMAI, K. N. PAUL;
UCLA, Los Angeles, CA

Abstract: Sex differences in the prevalence and severity of sleep impairments are well-documented and are often more pronounced under pathological conditions. One such condition is insomnia, a significant public health concern that disproportionately affects women and is frequently associated with stress. Sleep is regulated by a homeostatic mechanism that allows the body to recover from sleep loss during subsequent sleep opportunities. However, sex differences in recovery from sleep loss are often influenced by the stress associated with sleep deprivation. To investigate these sex differences, we characterized sleep architecture using electroencephalographic/electromyographic (EEG/EMG) recordings in the Four Core Genotype

(FCG) mouse model, which enables the dissociation of phenotypic sex from chromosomal sex, allowing precise manipulation of genetic and hormonal variables. This mouse line features four phenotypes (phenotypic males: XY^{Sry}, XX^{Sry}; phenotypic females XY^{-}, XX^{-}). After 24 hours of baseline recording, we subjected the mice to 1 hour of restraint stress or sleep deprivation (via gentle handling) during the light phase in a 12-hour light:12-hour dark cycle. We recorded recovery sleep following either sleep deprivation or restraint stress and compared sleep phenotypes across experimental groups. Initial findings have revealed that phenotypically male mice exhibit increased NREM sleep, increased REM sleep, and decreased wake amount (male n=15, female n=15; NREM p=0.009; REM p=0.008, wake p=0.03; repeated measures ANOVA; Tukey's post-hoc test) during the 18-hour recovery period following stress. Differences in REM sleep amount during recovery are influenced more by gonadal hormones (male n=15, female n=15, p=0.04; two-way ANOVA; paired t-test post-hoc), while differences in wake amount during recovery are influenced more by sex chromosome complement X or Y (male n=15, female n=15, p=0.05; two-way ANOVA; paired t-test post-hoc). After performing prepubertal gonadectomy (GDX) surgery at postnatal day 21, sex differences in recovery sleep were eliminated across all experimental groups, which suggests that gonadal hormones play an organizational role during development and are the causal factor behind these sex differences in adulthood.

Disclosures: M.R. Ralston: None. **M. Kahan:** None. **K. Choudhary:** None. **E. Lee:** None. **W. Kahende:** None. **K. Tamai:** None. **K.N. Paul:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.03/BB7

Topic: G.07. Biological Rhythms and Sleep

Title: A group of preoptic neurons involved in sleep regulation

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Abstract: Sleep is essential for the survival and well-being of all animals. However, our knowledge of the critical neuronal types and their brain-wide interactions involved in sleep regulation remains incomplete. Previous studies have pinpointed the hypothalamic preoptic area (POA) as a crucial region for sleep control, but the sleep-active and wake-active neurons are spatially and molecularly intermingled. This overlap complicates our ability to further understand and treat sleep disorders. Based on single-cell RNA-seq results from our lab, we identified a group of sleep-active (SA) neurons in the POA. Fiber photometry recordings confirmed that SA neurons were active during sleep and silenced during wakefulness. Activation of SA neurons promoted sleep, while their inhibition reduced it. Furthermore, disruption of the

SA gene led to reduced sleep, whereas intra-POA injection of the SA ligand (SALGD) increased sleep, indicating its critical role in sleep regulation. Therefore, we identified a neuronal population in the POA that played a key role in sleep regulation, with the SA gene contributing to sleep control, potentially offering a target for treating sleep disorders.

Disclosures: J. Zhao: None. **H. Cao:** None. **Z. Zhang:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.04/BB8

Topic: G.07. Biological Rhythms and Sleep

Title: The MCH neuronal system underlies brain clearance across sleep-wake states

Authors: *Y. ZHAO[1], Y. ZHAO[3], N. N. GUAN[3], Z. LIU[2], C.-X. HUANG[3], J. SONG[4]; [1]Tongji university, Shanghai, China; [2]Tongji university, shanghai, China; [4]Sch. of Med., [3]Tongji Univ., Shanghai, China

Abstract: Waste clearing system of the CNS constantly removes metabolic byproducts and toxins to maintain brain functions. Current research primarily highlights the specialized mechanisms underlying acceleration of glymphatic flow during sleep as opposed to wakefulness. However, the fundamental neuronal organization governing glymphatic dynamics and brain clearance across sleep and wake states remains incompletely understood.Here, we utilized larval zebrafish to simultaneously record neuronal activity and fluid (cerebrospinal and interstitial) dynamics in vivo, revealing that sleep-associated increase in neuronal synchrony are temporally coupled with enhanced fluid speed and waste removal. We identified hypothalamic melanin-concentrating hormone (MCH) neurons as a key neural substrate that orchestrates glymphatic dynamics and waste removal in an activity-dependent manner across sleep-wake states. While sparse and low-intensity activation of MCH neurons during wakefulness sustained baseline clearance, synchronized and high-intensity activity of them during sleep promoted global neuronal synchrony to boost glymphatic flow and efficient waste removal. Chemogenetic ablation of MCH neurons disrupted neural synchrony and largely impeded the fluid movement and waste removal. Notably, activation of the MCH system substantially potentiated the glymphatic flow and brain clearance during both states. Mechanistically, these effects are mediated via MCHR1 receptors distributed across multiple brain regions. Collectively, our findings establish the hypothalamic MCH system as a critical orchestrator of brain clearance, functioning across sleep and wake states, and provide new insights into state-dependent clearance mechanisms

Disclosures: Y. Zhao: None. **Y. Zhao:** None. **N.N. Guan:** None. **Z. liu:** None. **C. Huang:** None. **J. Song:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.05/BB9

Topic: G.07. Biological Rhythms and Sleep

Support: AMED JP21zf0127005
JSPS KAKENHI 18K14811
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JSPS KAKENHI 22K19552
JSPS KAKENHI 22K21351
JSPS KAKENHI 22H04918
JSPS KAKENHI 24H00671

Title: Molecular mechanisms of REM sleep regulation ~identifying protein-protein interactions of NALCN channel complex through BioID~

Authors: *H. YAMAMOTO[1,2,3,4], T. FUJIYAMA[1,3,4], D. SHONAI[3], S. KANNO[1,4], M. KAKIZAKI[1,4], S. H. SODERLING[3], H. FUNATO[5,1,4], M. YANAGISAWA[1,4];
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Abstract: Sleep is a ubiquitous behavior conserved both vertebrates and invertebrates. It is typically divided into rapid eye movement (REM) and non-REM sleep; however, REM sleep is unique to animals with highly developed brain structures. The molecular and cellular mechanisms underlying REM sleep regulation remain largely unknown. *Dreamless* mutant mice, which carry a single amino acid substitution (N315K) in the NALCN protein, exhibit abnormalities in REM sleep regulation (*Nature*, 2016). NALCN is a non-selective cation channel known to be involved in the circadian rhythm and respiratory regulation, and NALCN channel complex proteins such as UNC80, UNC79, and FAM155A have been identified. However, the components of NALCN channel complex and molecular mechanisms of NALCN regulation in REM sleep homeostasis remain unknown. In this study, we aim to elucidate the molecular mechanisms of REM sleep regulation mediated by NALCN by conducting a comprehensive search for protein-protein interactions of NALCN channel complex using a biotin-based proximity labeling technique, BioID. We generated two knock-in mice in which NALCN and UNC80 fused to multiple-tags consisting of HA-tag, strepIII-tag and promiscuous mutant biotin ligase BirA, that enables parallel analyses of both *in vivo* BioID (iBioID) and conventional affinity purification-mass spectrometry. Western blot analysis of purified streptavidin-precipitations detected a marked increase in biotinylated proteins and the presence of epitope-tagged bait proteins at expected molecular masses. LC-MS/MS analysis of these lysates identified 184 proteins, including known interacting proteins, that were enriched 1.5-fold compared to control mice and were common proximity proteins of both iBioID of NALCN and UNC80. The hypergeometric p-value of these data indicates a highly significant overlap. Gene

Ontology (GO) term analysis classified the identified proteins by subcellular localization, revealing many localized to mitochondria and vesicles, in addition to the plasma membrane. Notably, the common proximity proteins included molecules known to be involved in sleep regulation, which are predicted to be upstream signals of NALCN channel complex. Our findings identify previously uncharacterized interacting proteins that may regulate NALCN activity. In future studies, we plan to further select proteins, generate genetically modified mice with altered function of these targets, and conduct sleep analysis to elucidate the relationship with REM sleep regulation.

Disclosures: H. Yamamoto: None. **T. Fujiyama:** None. **D. Shonai:** None. **S. Kanno:** None. **M. Kakizaki:** None. **S.H. Soderling:** None. **H. Funato:** None. **M. Yanagisawa:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.06/BB10

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant R01NS078168
NIH Grant U19NS128613
American Heart Association Predoctoral fellowship 25PRE1371919
NIH Grant T32GM108563
NIH Grant F31ES036154

Title: Interneuron control of arteriole dilation during sleep

Authors: *F. SALEHI SHAHRBABAKI, J. FLORES, D. GREENAWALT, P. J. DREW; The Pennsylvania State Univ., State College, PA

Abstract: Neural activity plays a crucial role in regulating arterial dynamics in the brain through neurovascular coupling. During sleep, arterial dilations are more prominent compared to wakefulness. Vascular changes create mechanical forces in the perivascular space and pump cerebrospinal fluid (CSF) in the brain, potentially removing waste, making understanding the mechanism controlling these vascular dynamics important. However, the specific neuronal cell type controlling arteriole changes during sleep has not been fully elucidated. Activation of parvalbumin (PV) GABAergic interneurons drives biphasic hemodynamics responses, via the activation of type I neuronal nitric oxide synthase (nNOS) neurons through substance P (SP receptors. Approximately half of PV interneurons produce SP, which is encoded by the Tachykinin-1 (Tac1) gene. This suggests a critical role for substance P in controlling arterial diameter changes during sleep. To investigate this, we characterized the activity of Tac1-expressing and PV interneurons using GCaMP, while imaging cortical hemodynamics using wide-field optical imaging in head-fixed mice. We recorded across sleep and wake states, and the responses to whisker stimulation. The activity of both Tac1 and PV expressing interneurons

significantly increased both to whisker stimulation and during REM and NREM sleep. In both cases their activity was correlated with vasodilation. Optogenetic stimulation of these neurons induced a transient vasoconstriction during stimulation, followed by a robust vasodilation post-stimulation, which was influenced by the arousal state of the mouse. Together, these findings suggest that Tac1 and PV neurons modulate vascular dynamics during both sleep and wake.

Disclosures: F. Salehi Shahrbabaki: None. **J. Flores:** None. **D. Greenawalt:** None. **P.J. Drew:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.07/BB11

Topic: G.07. Biological Rhythms and Sleep

Support: VA Grant 2I01BX004693-05A1
NIA Grant R01-AG076030
NINDS Grant T32-5NS082145-12

Title: The Orexin System: A Target for the Treatment of Stress-Induced Sleep Alterations in a Rodent Model of PTSD

Authors: *K. LILLY[1], S. M. PEREZ[2], D. J. LODGE[3];
[1]UT Hlth. San Antonio, SAN ANTONIO, TX; [2]Pharmacol., [3]UTHSCSA, San Antonio, TX

Abstract: Post-traumatic stress disorder (PTSD) is a debilitating condition that may develop when a person experiences an intense traumatic event and that often co-occurs with other psychiatric disorders. A significant and often overlooked challenge for patients with PTSD is disrupted sleep which can severely affect quality of life and, in turn, exacerbate PTSD symptoms. We have previously shown that Suvorexant, an FDA-approved dual OXR antagonist, reverses both stress-induced dopamine dysfunction and corresponding deficits in psychosis-like behaviors. Given the known role of orexins in the regulation of sleep and the ability of Suvorexant to enhance REM sleep, we hypothesized that orexin receptor antagonists could also improve stress-induced sleep alterations. Indeed, a randomized, double-blind, placebo-controlled trial reported beneficial effects of Suvorexant on sleep in people with PTSD; however, the relative role of the distinct orexin receptors in this process is unknown. Sleep architecture can be directly measured in rodents using wireless electro-encephalography (EEG). In rats, Suvorexant and other selective antagonists, SB334867 (OX1R) and EMPA (OX2R), were given systemically (10 mg/kg *i.p.*) before and after stress. EEG recordings captured 24 hours of wideband signal that was analyzed via cluster-based sleep scoring software. All controls were within-subject with counter-balanced drug order. We found that inescapable foot-shock stress using the self-enhanced fear learning (SEFL) paradigm produced substantial reductions in REM sleep and significantly increased the number of arousals from both NREM and REM sleep. The SEFL

model also drives aberrant physiological and behavioral phenotypes consistent with core dimensions of psychosis and PTSD in humans, making it a relevant model for these experiments. Here, we posited that targeting the orexin system with OXR antagonists, specifically those acting on OX1R, would alleviate SEFL-induced disruptions in REM sleep. Indeed, all OXR antagonists improved REM density without altering NREM sleep and restored sleep architecture following stress. This suggests that both OX1R and OX2R play a role in regulating REM sleep. Taken together, these data indicate that targeting the orexin system is relevant for the treatment of stress-induced sleep disruptions, particularly in PTSD patients.

Disclosures: K. Lilly: None. **S.M. Perez:** None. **D.J. Lodge:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.08/BB12

Topic: G.07. Biological Rhythms and Sleep

Title: Drug effects on sleep architecture and qEEG: a cross-species comparison in mice, rats and dogs.

Authors: J. R. HUXTER[1], S. KELLY[3], A. VARDHAN[5], R. PERCIVAL[3], *C. DE RIVERA[3], S. THEVARKUNNEL[4], L. GIGGINS[2], K. MARSZALEK[6], M. DOMZALSKA[7], E. SOKOLOWSKA[8];
[1]Transpharmation Ltd. UK, Haverhill, United Kingdom; [2]Transpharmation Ltd. UK, Kent, United Kingdom; [3]Transpharmation Canada Ltd., Fergus, ON, Canada; [4]Transpharmation Canada Ltd., Toronto, ON, Canada; [5]Transpharation Ltd, Fergus, ON, Canada; [6]Transpharmation Ltd., Warsaw, Poland; [7]Transpharmation, Ltd., Olsztyn, United Kingdom; [8]Transpharmation Poland Sp. z o.o, Warszawa, Poland

Abstract: The mechanisms controlling sleep and neuronal network oscillations are highly conserved across mammalian species, and are therefore ideal assays for translational research supporting drug discovery in both animals and humans. The ease with which sleep and electroencephalography (EEG) can be measured also makes them suitable for use as pharmacodynamic biomarkers both clinically and preclinically. The current work investigates the similarities between drug induced changes in sleep and EEG in mice, rats and dogs, using a comparable EEG montage and a single, automated data analysis workflow. Eleven mice, nine rats and five dogs were implanted with wireless telemetry for the recording of EEG, muscle-tone (EMG), locomotor activity and body temperature. Animals were dosed with modafinil (200 mg/kg p.o.) and recordings were made for 24 hours. Automatic sleep-wake scoring was performed, and the hourly distribution of wakefulness, NREM-sleep and REM-sleep were analysed. Short-time FFT was used for state-dependent assessment of EEG frequency bands over time. The most salient responses to modafinil were common to all species. Activity and body temperature were upregulated in both species. Wakefulness was significantly increased in mice

(p=0.0341) rats (p=0.0006) and dogs (p=0.0043) for up to 8 hours after dosing. Both REM and non-REM sleep were suppressed. EEG effects were most pronounced during wakefulness, and there was a clear increase in total-gamma (30-100Hz) which was significant in mice ($F_{1.50,13.52}$=5.59 p=.023), and high-gamma which was significant in rats ($F_{1,8}$=32.84 p<.001) and dogs ($F_{1,4}$=20.61 p=.010). These results indicate that, when attention is paid to the electrode type, the positioning on the skull, and the way in which the data is processed, highly comparable sleep and EEG responses to drugs can be detected across species. Sleep-EEG is, therefore, a powerful tool for confirming target engagement, pharmacodynamics and equivalent doses in both clinical and preclinical research.

Disclosures: J.R. Huxter: A. Employment/Salary (full or part-time): Transpharmation Ltd. **S. Kelly:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **A. Vardhan:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **R. Percival:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **C. de Rivera:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **S. Thevarkunnel:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **L. Giggins:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **K. Marszalek:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **M. Domzalska:** A. Employment/Salary (full or part-time): Transpharmation Ltd. **E. Sokolowska:** A. Employment/Salary (full or part-time): Transpharmation Ltd.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.09/BB13

Topic: G.07. Biological Rhythms and Sleep

Title: Role of Primary cilia of the Nucleus accumbens core in time dependent and motivated behaviors

Authors: *R. VAKIL MONFARED[1], A. ALACHKAR[2];
[1]Univ. of California Irvine, Irvine, CA; [2]Pharmaceut. Sci., Univ. of California, Irvine, Irvine, CA

Abstract: Primary cilia are hair-like organelles present on most neurons in the mammalian brain. Functioning as cellular antennae, they sense external signals and regulate various cellular functions. Many of the signals that primary cilia receive have circadian fluctuations like MCH neuropeptides. We have shown circadian oscillations in the expression of cilia-related genes across different brain regions in primates and mice. We have also shown that certain brain regions, including the nucleus accumbens core (NAcc), demonstrated circadian oscillations in cilia length. The NAcc plays a critical role in modulating motivational and circadian behaviors. Both D1 and D2 dopamine receptors are involved in mediating the effects of dopamine on social interactions as well as sleep. Based on this discovery, we are now investigating whether removing primary cilia from the NAcc alters these behaviors. To achieve this, we are using

IFT88 floxed mice, in which the IFT88 gene—essential for cilia structure—can be selectively knocked out using Cre recombinase. We inject AAV-Cre viruses into the NAcc to ablate primary cilia. Our experimental groups include wild-type controls, mice with total neuronal cilia removal in the NAcc, and two groups with cell-type-specific cilia ablation in either D1 or D2 neurons using neuron-specific AAV-Cre constructs.One week after recovery, we assess circadian behaviors such as food intake, wheel running, and sleep patterns using EEG recordings, followed by tests of motivational behavior, including conditioned place preference with cocaine, sucrose preference, and social interaction assessments. Our results from the whole-cilia removal group show reduced wheel-running activity and impairments in social interaction and novelty recognition.These findings could offer novel insights into cilia's role in behavioral regulation and uncover mechanisms underlying social and circadian disturbances in disorders such as autism and schizophrenia, potentially positioning primary cilia as therapeutic targets.

Disclosures: R. Vakil monfared: None. **A. Alachkar:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.10/BB14

Topic: G.07. Biological Rhythms and Sleep

Title: Rationale and Study Design of the Phase-3 Clinical Development Program for Samelisant (SUVN-G3031) in the Treatment of Excessive Daytime Sleepiness in Patients with Narcolepsy

Authors: *V. GOURA, V. GOYAL, A. K. SHINDE, P. JAYARAJAN, J. RAVULA, S. JETTA, V. PALACHARLA, R. SUBRAMANIAN, R. BADANGE, R. NIROGI;
Suven Life Sci. Ltd., Hyderabad, India

Abstract: Samelisant (SUVN-G3031) is a potent and selective inverse agonist of the histamine H_3 receptor. In preclinical studies, Samelisant demonstrated both wake-promoting and anticataplectic effects in animal models relevant to narcolepsy. Samelisant was evaluated in orexin knockout mice and orexin-B saporin (SAP)-lesioned rats to assess its effects on sleep-wake patterns and cataplexy using electroencephalography. In orexin knockout mice, Samelisant significantly increased the time spent in wake period and reduced cataplexy-like episodes. Similarly, in orexin-B SAP-lesioned rats, it demonstrated wake-promoting effects and led to a significant decrease in direct transitions from wakefulness to rapid eye movement sleep, a hallmark of narcolepsy. Samelisant was evaluated as a monotherapy in a Phase-2 proof-of-concept, double-blind, randomized controlled trial (NCT04072380) for the treatment of excessive daytime sleepiness (EDS) in patients with narcolepsy, with or without cataplexy. At Day 14, Samelisant demonstrated a statistically significant and clinically meaningful reduction of 2.1 points in Epworth Sleepiness Scale (ESS) total score compared to placebo ($p < 0.024$). The improvement in the primary endpoint was further supported by statistically significant benefits on key secondary endpoints, including the Clinical Global Impression-Severity, Patient Global

Impression of Change, and Clinical Global Impression of Change. Samelisant was found to be safe and well tolerated in the study population. Samelisant is planned to be investigated in a Phase-3 clinical study for the treatment of EDS in patients with narcolepsy. This study is a placebo-controlled, double blind randomized, parallel-group study. The study will recruit about 216 narcolepsy patients with an established diagnosis of narcolepsy. After a screening period of 4-weeks, eligible patients will follow a titration schedule to gradually increase their dose of the investigational product for initial four weeks followed by stable dose treatment for additional eight weeks. The primary endpoint of the study is change from Baseline to Week 12 in total ESS score. Secondary endpoints are change from Baseline to Week 12 in Maintenance of Wakefulness Test score, CGI-S score and Narcolepsy Severity Scale score. Phase-3 study will be initiated in H2 2025 and results from this study may inform the therapeutic utility of Samelisant for the treatment narcolepsy.

Disclosures: V. Goura: A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **V. Goyal:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **A.K. Shinde:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **P. Jayarajan:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **J. Ravula:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **S. Jetta:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **V. Palacharla:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **R. Subramanian:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **R. Badange:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment. **R. Nirogi:** A. Employment/Salary (full or part-time): SUVEN LIFE SCIENCES LTD, Full-time Employment.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.11/BB15

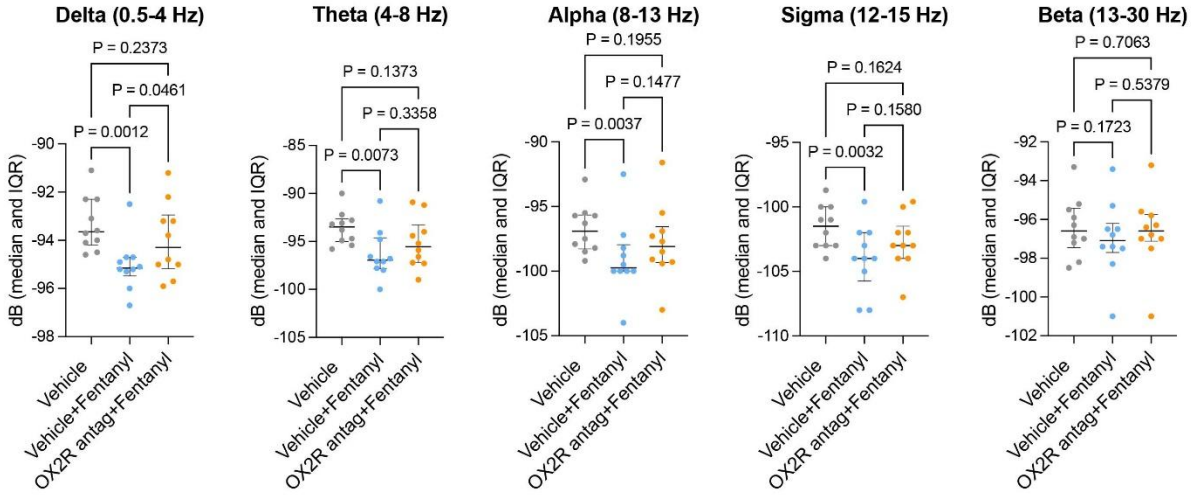
Topic: G.07. Biological Rhythms and Sleep

Support: University of Tennessee, Knoxville

Title: Fentanyl-induced sleep disruption in C57BL/6J mice is characterized by decreases in cortical EEG power that are reversed by an orexin-2 receptor antagonist

Authors: *N. W. COOPER[1], D. ZEBADÚA UNZAGA[1], M. BEEBE[2], R. LYDIC[1], H. A. BAGHDOYAN[1];
[1]Psychology and Neurosci., [2]Biomed. Engin., Univ. of Tennessee, Knoxville, TN

Abstract: Many laboratories are exploring the use of polypharmacy with the goal of providing pain relief without causing sleep disruption. Fentanyl disrupts EEG power in humans (10.1093/pnasnexus/pgac158) and in C57BL/6J (B6) mice fentanyl disrupts sleep and EEG power (10.1152/jn.00266.2021). Preliminary data in B6 mice show that fentanyl-induced sleep disruption is partially mitigated by pretreatment with the orexin-2 receptor (OX2R) antagonist JNJ10397049 (10.1016/j.jpet.2025.103472). The objective of this ongoing study is to evaluate the hypothesis that the OX2R antagonist reverses fentanyl-induced changes in EEG power during fentanyl-induced wakefulness. These studies were IACUC reviewed and approved. Adult, male B6 mice (n=10) were housed in a 12:12 light/dark cycle. EEG and EMG recordings were obtained from mice in their home cages via telemetry (DSI HD-X02). Injections (0.3 mL) were made 15 min before lights off. Using a within-subject, repeated measures design, mice received the vehicle control (15% DMSO in DI water) or the OX2R antagonist (10 mg/kg) 15 min before receiving a sleep-disruptive dose of fentanyl (0.3 mg/kg) (10.1096/fasebj.2021.35.S1.01706). Recordings of sleep/wake states began immediately after fentanyl injection. Data were analyzed for the first 30 min after fentanyl administration. Repeated measures one-way ANOVA revealed a significant treatment main effect (P<0.01) on delta, theta, alpha, and sigma frequency bands (eta squared values of 0.23, 0.21, 0.13, and 0.18, respectively). There was no treatment main effect on beta power (Fig). Tukey's multiple comparisons test showed that fentanyl significantly (P<0.01) decreased EEG power in delta, theta, alpha, and sigma frequency bands (Cohen's d values ≥ 1), and that the OX2R antagonist reversed the fentanyl-induced decreases in EEG power for those 4 frequency bands (P = in Fig). In conclusion, these findings support the hypothesis. Ongoing analyses are quantifying the time-course of 1) fentanyl-induced decreases in EEG power, and 2) reversal of these changes by the OX2R antagonist.



Disclosures: N.W. Cooper: None. **D. Zebadúa Unzaga:** None. **M. Beebe:** None. **R. Lydic:** None. **H.A. Baghdoyan:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.12/CC1

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant 2R15NS101692-02A1
Skidmore College Faculty/Student Summer Research Award

Title: Interactions of octopamine neurons and astrocytes in sleep regulation in Drosophila melanogaster

Authors: Y. SU[1], *C. G. VECSEY[2];
[1]Skidmore Col., Saratoga Springs, NY, ; [2]Neurosci., Skidmore Col., Saratoga Springs, NY

Abstract: Astrocytes are multifunctional glial cells that have recently been implicated in sleep homeostasis. In the fruit fly *Drosophila melanogaster*, experimental activation of astrocytes over a 24-hour period leads to a gradual increase in sleep, followed by a rebound phase once activation ceases. Octopamine (OA), the invertebrate analog of norepinephrine, modulates diverse behaviors such as feeding initiation, locomotion, aggression, and courtship. Moreover, activation of TDC2 neurons, which release OA, promotes wakefulness during nighttime. This study aimed to determine whether OA signaling interacts with astrocyte activation to regulate sleep and to identify the neuronal components involved in this interaction. We employed thermogenetic and optogenetic approaches to manipulate neuronal activity. Our findings suggest that astrocytes and TDC2 neurons have counterbalancing roles; specifically, astrocyte activation can attenuate the arousal effects of OA. However, astrocytes and TDC2 neurons do not appear to function directly upstream or downstream of each other, suggesting the involvement of additional cells in this signaling pathway. Therefore, we next tested whether insulin-producing cells might be a downstream target of TDC2 neurons. To do this, we employed the P2X2 system to activate TDC2 neurons using ATP treatment, while simultaneously monitoring calcium levels using the fluorescence indicator GCaMP to assess the activity of insulin-producing cells. Our data suggest that insulin-producing cells receive excitatory synaptic signals from TDC2 neurons; however, their specific roles in the TDC2-astrocyte interaction and sleep regulation remain to be clarified.

Disclosures: Y. Su: None. **C.G. Vecsey:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.13/CC2

Topic: G.07. Biological Rhythms and Sleep

Support: R01EY027779
R01EY027779-S1
R01EY032080
Research to Prevent Blindness

Title: Suvorexant helps protect against retinal neurovascular defects in db/db mice

Authors: *R. K. SODHI;
Ophthalmology, Indiana University, Indianapolis, Indianapolis, IN

Abstract: Purpose: Orexin is a neuropeptide primarily produced by the hypothalamus, playing a crucial role in regulating appetite, maintaining wakefulness and alertness, as well as managing glucose levels in the body. In a clinical setting, suvorexant, which inhibits both orexin receptors, is used in the insomnia treatment. Notably, the timed administration of suvorexant leads to a reduction in blood glucose levels. High blood glucose is a significant factor contributing to diabetic retinopathy (DR), which is the most common complication of diabetes. By employing an animal model of type 2 diabetes (T2D), we hypothesized that treatment with suvorexant could ameliorate the neurovascular impairments associated with T2D. **Methods:** Seven-week-old male diabetic mice (db/db) received daily treatments of suvorexant (30 mg/kg, i.p.) at zeitgeber time 0-2 for 12 weeks. Body weight and food intake were recorded weekly. Glucose diurnal rhythm was conducted during 8th week of treatment. Glucose and insulin tolerance tests were conducted near the end of the treatment period. Visual assessments were performed using electroretinograms, optokinetic response tests, and fluorescein angiography after 12 weeks. Following euthanasia, the retina was extracted, and qPCR was conducted for retinal orexin signaling and retinal inflammation. **Results:** Diabetic mice treated with suvorexant exhibited a decline in body weight and food consumption, accompanied by improved glucose tolerance, compared to those receiving a vehicle treatment. The treatment with suvorexant enhanced visual acuity and resulted in an increase in the largest vein diameter and vascular area, while reducing the avascular area. In suvorexant-treated mice, there was a reduction in the amplitudes of the scotopic and photopic b-wave. Correspondingly, we observed elevated levels of orexin signaling (PLCG1 and PKCB) and inflammatory markers (TNF-α and IL-1β) in the retinas of diabetic mice, which were significantly decreased by the administration of suvorexant. **Conclusion:** Our findings suggest that suvorexant may be explored further as a potential treatment strategy for DR.

Disclosures: R.K. Sodhi: None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.14/CC3

Topic: G.07. Biological Rhythms and Sleep

Title: Sleep enhance glymphatic flow and brain clearance through REM-associated activation of MCH neurons

Authors: *C. WANG[1], Y. ZHAO[2], W. MENG[1], N. N. GUAN[2], C.-X. HUANG[2], J. SONG[3];
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Abstract: Brain clearance has been closely associated with distinct brain states, with glymphatic clearance shown to be enhanced during sleep or anesthesia. While certain sleep feature-related mechanisms have been implicated in regulating glymphatic function, the role of REM sleep remains poorly understood. However, a central question remains as to whether glymphatic clearance is fundamentally directed by a specific neuronal system within the central nervous system, or exclusively activated by sleep or anesthesia. We hypothesize that glymphatic clearance is orchestrated by a dedicated neuronal system, whose function efficacy is state-modulated across distinct brain states.Here, we investigated brain clearance under three natural states (nighttime, daytime and rebound sleep) characterized by distinct sleep-wake patterns. Both the cerebrospinal fluid (CSF)-to-interstitial fluid (ISF) perfusion and waste removal were enhanced alongside increased REM sleep and the hypothalamic melanin-concentrating hormone (MCH) neuron activity. REM deprivation or chemogenetic inhibition of MCH neurons impaired glymphatic perfusion and waste removal. Notably, optogenetic activation of MCH neurons substantially potentiated glymphatic flow and brain clearance during both wakefulness and sleep. Mechanistically, MCH neuron activation enhanced neuronal synchronization across multiple brain regions. Our study demonstrated that the REM-associated hypothalamic MCH system acts as a neuronal substrate of glymphatic flow and brain clearance, operating across wake and sleep states, and provide new insights into how REM sleep contributes to clearance mechanisms.

Disclosures: C. Wang: None. **Y. Zhao:** None. **W. Meng:** None. **N.N. Guan:** None. **C. Huang:** None. **J. Song:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.15/CC4

Topic: G.07. Biological Rhythms and Sleep

Support: JSPS Overseas Research Fellowships
R01NS118856
R01NS073613
R01NS122589

Title: Gabaergic projections from the dmh to the poa promote wakefulness in mice

Authors: *F. ASANO[1], C. ANACLET[1], E. ARRIGONI[2], P. FULLER[1];
[1]Dept. of Neurolog. Surgery, Univ. of California, Davis, Davis, CA; [2]Neurol., BIDMC and Harvard Med. Sch., Boston, MA

Abstract: To survive, animals must eat, drink, reproduce, and sleep. Under conditions of food scarcity, animals prioritize foraging over sleep and adjust their sleep cycles to align with periods when food is available. Previous studies have shown that food deprivation increases time spent awake during both active and rest periods. Food deprivation has also been shown to enhance the excitability of dorsomedial hypothalamus (DMH) GABAergic neurons. These neurons project to the preoptic area (POA), including the ventrolateral preoptic area (VLPO), which contains GABAergic neurons that express galanin, are active during sleep, and promote sleep. We propose that DMH GABAergic neurons may promote arousal during fasting by inhibiting the sleep-promoting neurons of the VLPO. When we deprived animals of food for 24 hours (with food removed at light-off), we observed increased wakefulness and disrupted sleep. Specifically, we found that mice did sleep during total food deprivation, but delta power decreased early in the deprivation period, around 3 hours, and remained reduced throughout the entire 24-hour period. This was accompanied by an increase in microarousals, which began to rise around 9 hours after food removal. A significant increase in overall wakefulness was observed only after 21 hours of food deprivation (n = 9 mice). Optogenetic stimulation of DMH GABAergic terminals in the preoptic area, just above the VLPO, produced immediate transitions from non-REM sleep to wakefulness. Food-deprived mice exhibited a higher probability of awakening in response to optogenetic stimulation of DMH GABAergic inputs to the VLPO. Our results indicate that while increased wakefulness in response to food deprivation takes several hours to manifest, sleep is disrupted much earlier, as evidenced by reduced delta power and frequent microarousals occurring just a few hours after food removal. The input from DMH GABAergic neurons to the VLPO rapidly induces arousal from sleep, with a greater effect observed in food-deprived animals. We propose that DMH GABAergic neurons projecting to the POA and VLPO play a crucial role in promoting wakefulness during fasting.

Disclosures: F. Asano: None. **C. Anaclet:** None. **E. Arrigoni:** None. **P. Fuller:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.16/CC5

Topic: G.07. Biological Rhythms and Sleep

Support: Research Council of Finland Grant 356491

Title: Distinct Effects Of Conventional And Rapid-Acting Antidepressants On Sleep Structure

Authors: *L. ILKKA, H. ANTILA;
Univ. of Helsinki, Helsinki, Finland

Abstract: While the involvement of monoamines in both sleep regulation and antidepressant action is well established, the interplay between the two remains unclear. A prevalent hypothesis suggests that antidepressants mediate their therapeutic effects by inducing neuronal plasticity.

Sleep is critical for neuronal plasticity, yet the effects of antidepressants on sleep structure and function have been understudied. Recently, infraslow (0.02 Hz) rhytmic fluctuations in monoaminergic neuron activity during NREM sleep have been identified. Similar 0.02Hz infraslow rhythm can be observed in EEG sigma power, consisting mainly of sleep spindles. Monoaminergic activity during sleep has powerful control over the sleep microarchitecture, as well as NREM to REM sleep transitions. As monoaminergic systems are the main targets of antidepressant drugs, this study investigates whether different pharmacological agents used to treat depression have distinct effects on sleep microstructure through their influence on monoaminergic activity during sleep. We used 24-hour EEG recordings in female mice to examine the effects of SSRIs (selective serotonin reuptake inhibitors) and rapid-acting antidepressants on sleep. Compared to SSRIs, rapid-acting antidepressants show more subtle changes in sleep architecture during the first 24 h post-injection. Analysis of recordings from days 7 and 14 will assess the persistence of these effects. In a parallel experiment, we used male mice expressing noradrenaline sensor GRABne to record noradrenaline activity in the basolateral amygdala and anterior cingulate cortex during sleep. We analyzed noradrenaline dynamics following injection of the same antidepressant compounds and found drug-specific alterations in noradrenaline levels during NREM sleep, correlating with changes in sleep structure.
These findings support the idea that antidepressants with distinct pharmacological profiles differentially affect sleep, which may underlie differences in their ability to induce brain plasticity.

Disclosures: L. Ilkka: None. **H. Antila:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.17/CC6

Topic: G.07. Biological Rhythms and Sleep

Title: Improvement of hypersomnia symptoms and development of novel hypersomnia therapeutics focusing on the histamine-metabolizing enzyme HNMT.

Authors: K. HIRANO[1], ***T. YOSHIKAWA**[2], F. NAGANUMA[2];
[1]Dept. of Neuropharmacology, Grad. Sch. of Medicine, Hokkaido Univ., Sapporo-shi Kita-ku, Japan; [2]Hokkaido Univ., Sapporo, Japan

Abstract: Brain histamine plays a crucial role in promoting wakefulness. Clinical studies have shown that histamine concentrations in the cerebrospinal fluid are reduced in patients with narcolepsy, regardless of their orexin levels. These findings have led to the hypothesis that enhancing brain histamine could serve as a therapeutic strategy for treating hypersomnia. There are several approaches to increasing brain histamine levels. For example, histamine H3 receptor inverse agonists—such as pitolisant, an approved treatment for narcolepsy—regulate histamine release. Another approach is to inhibit histamine N-methyltransferase (HNMT), the enzyme

responsible for histamine degradation.Our previous research demonstrated that HNMT plays a key role in regulating brain histamine levels. In the present study, we assessed the efficacy of metoprine, an HNMT inhibitor, in multiple mouse models of hypersomnia. Administration of metoprine significantly increased wakefulness in three distinct models: narcolepsy, idiopathic hypersomnia, and Parkinson's disease, which exhibits hypersomnia during the active phase. This wake-promoting effect was abolished by pyrilamine, a histamine H1 receptor antagonist, confirming the involvement of histaminergic mechanisms.Moreover, metoprine exhibited a stronger arousal effect than currently approved treatments. In narcoleptic mice, it reduced the frequency of cataplexy and shortened the latency to its onset more effectively than pitolisant. In idiopathic hypersomnia model mice, metoprine induced greater wakefulness than an orexin agonist.These findings suggest that HNMT inhibitors may be effective across a range of hypersomnia conditions. However, current HNMT inhibitors, including metoprine, are limited by suboptimal specificity and poor brain penetrance, highlighting the need for novel compounds with improved pharmacodynamic and pharmacokinetic profiles. To address this, we conducted high-throughput screening to identify new HNMT inhibitor candidates. Several promising compounds have been identified and are currently undergoing counter-assays to confirm their specificity. Additionally, we are using crystal structure analysis to further characterize select candidates.These findings underscore the therapeutic promise of HNMT inhibition for hypersomnia and emphasize the importance of developing next-generation inhibitors with enhanced specificity and brain penetrance.

Disclosures: K. Hirano: None. **T. Yoshikawa:** None. **F. Naganuma:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.18/CC7

Topic: G.07. Biological Rhythms and Sleep

Support: 5R01AG062398
5R01NS114780

Title: Sleep deprivation selectively reactivates hippocampal CA1 pyramidal neurons

Authors: *Y. WANG[1], E. WALSH[4], J. RESCH[2], L. C. LYONS[5], T. ABEL[3];
[1]Neurosci. and Pharmacol., Univ. of Iowa, IOWA CITY, IA; [2]Univ. of Iowa, Iowa City, IA, ; [3]Dept. of Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA; [4]Neurol., Washington Univ. in St. Louis, St. Louis, MO; [5]Dept. of Biol. Science, Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: Sleep supports a variety of physiological processes ranging from metabolism to immune system homeostasis and plays a critical role in cognition and memory. A brief period of sleep loss impairs memory, particularly hippocampus-dependent memories, and alters molecular

signaling and synaptic plasticity in the hippocampus. Studies have shown that sleep deprivation (SLD), alters neuronal activation as indicated by broad changes in gene expression signatures and by the altered expression of c-Fos, an immediate early gene (IEG) that functions as a molecular marker of neuronal activity. In the present study, we examined hippocampal subregion-specific c-Fos induction patterns. We find that CA1 pyramidal neurons exhibit the most robust c-Fos induction after SLD. Within CA1, the proximal region where spatial information is processed and encoded shows higher c-Fos levels after SLD as compared to the distal region. Using an activity-driven ribosomal tagging system and a repeated SLD model, we identify a specific population of excitatory neurons in area CA1 reactivated by repeated SLD. This approach also enables analysis of individual gene expression from c-Fos+ neurons to identify specific molecular signatures of neurons sensitive to repeated sleep loss. In summary, our study provides a detailed view of the activation of hippocampal neurons after SLD, revealing a subset of CA1 pyramidal neurons having higher sensitivity to the effect of sleep loss and laying the groundwork for further research to investigate molecular changes in neurons specifically impacted by repeated sleep loss.

Disclosures: Y. Wang: None. **E. Walsh:** None. **L.C. Lyons:** None. **T. Abel:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.19/CC8

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R21XA276027
NIH R21CA276027-02

Title: Serum ghrelin concentrations vary across tumor progression: Implications for hypocretin/orexin neuronal activity and sleep

Authors: *B. D. ELLIOTT, J. C. WALTON, B. M. CARNEY, R. J. NELSON, A. C. DEVRIES;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Altered sleep is common for individuals with breast cancer and is associated with negative health outcomes. We have previously demonstrated that mice with mammary tumors experience altered sleep in late tumor progression, which is associated with increased activity of wake-promoting hypocretin/orexin (HO) neurons. We further observed increased concentrations of serum ghrelin and identified GHRL mRNA within the tumor. As HO neurons express the receptor for ghrelin, this suggests that ghrelin produced by the tumor may be functionally relevant for altered neuronal activation and subsequent sleep disturbance. Additional work to understand the role of ghrelin on sleep revealed no differences in serum concentrations when tumor size was held constant at the time of measurement, as opposed to our earlier studies in

which time (days post inoculation) was held constant. Thus, the goal of the present study is to determine if serum ghrelin concentrations vary as a function of tumor size. We hypothesize that ghrelin concentrations increase across tumor progression. To test this, adult female Balb/c mice were assigned to one of three groups: control (no tumor), 67NR (ghrelin-producing) tumor, or 1G9 (ghrelin knockout) tumor. On day 0, mice received bilateral 100 µl orthotopic injections of either cancer cell line ($1.0x10^5$ cells/injection) or a vehicle (DMEM) into the fourth and ninth inguinal mammary glands. Starting on day 15, tumor size was measured bi-daily with calipers; food and body mass were recorded. Repeated blood sampling took place on days 15, 20, and 25 and total ghrelin was determined via ELISA. Our results revealed that ghrelin was significantly increased on Day 20 in mice with 67NR tumors relative to control and 1G9 tumor-bearing mice. There were no differences in ghrelin between groups on Days 15 or 25, which may indicate a compensatory downregulation of native ghrelin in mice bearing 67NR tumors by late tumor progression. These data suggest that ghrelin may not be driving sleep disruptions observed in late tumor development. Thus, we next sought to determine the direct role of HO neurons on sleep. We hypothesize that manipulating neuronal activity will result in sleep-wake changes. To test this, mice were injected with either Gq or Gi DREADDs to activate or inhibit HO neuronal activity, respectively. Sleep data, recorded via the PiezoSleep Behavioral Tracking System, will be reported. We predict that upregulating HO activity will increase wake and disrupted sleep similar to that observed in tumor-bearing mice. Together, the results from these studies should contribute to our understanding of mechanisms underlying altered sleep in individuals with mammary cancer.

Disclosures: B.D. Elliott: None. **J.C. Walton:** None. **B.M. Carney:** None. **R.J. Nelson:** None. **A.C. DeVries:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.20/CC9

Topic: G.07. Biological Rhythms and Sleep

Support: R35 NS122172

Title: Melatonin promotes sleep by suppressing responses to visual stimuli via MT1 receptors

Authors: *O. ELIOPOULOS, A. J. HILL, J. EMTAGE, G. OIKONOMOU, D. PROBER; Caltech, Pasadena, CA

Abstract: Sleep is an evolutionarily conserved behavioral state whose regulation remains poorly understood. A classical model postulates that sleep is mediated by two processes: a homeostatic process that increases sleep propensity with time spent awake, and a circadian process that determines when most sleep occurs during the 24-hour circadian cycle. While several factors have been shown to mediate homeostatic regulation of sleep in a partially redundant manner,

circadian regulation of sleep is abolished in zebrafish that lack melatonin, indicating that melatonin alone acts downstream of the circadian clock to mediate circadian regulation of sleep in this diurnal species. However, the mechanisms through which melatonin promotes sleep are unclear. In this study, we performed genetic loss-of-function experiments where we found that melatonin acts through the MT1 receptor paralogs *mtnr1aa*, *mtnr1ab*, and *mtnr1al*, and not via the MT2 receptor paralogs, to maintain normal night-time sleep levels, and for sleep that is induced by treatment with exogenous melatonin. We found that these receptors are highly expressed in neurons that receive and process visual information. Thus, we subjected melatonin-treated wild-type fish and melatonin-deficient *aanat2* mutant fish to behavioral assays that tested their responsiveness to visual stimuli. These experiments revealed that melatonin promotes sleep by suppressing behavioral responses to visual stimuli. These results indicate that circadian regulation of sleep by melatonin signaling is implemented, at least in part, by suppression of responses to environmental stimuli, which is a key feature of sleep.

Disclosures: O. Eliopoulos: None. **A.J. Hill:** None. **J. Emtage:** None. **G. Oikonomou:** None. **D. Prober:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.21/CC10

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant R35 NS122172

Title: Mutation of stk32a reveals link between sleep homeostasis and suppression of sensory and motor systems

Authors: *J. EMTAGE[1,2], S. TRAN[2], M. LECOEUCHE[4], G. OIKONOMOU[3], A. ANDREEV[5], M. B. AHRENS[6], D. PROBER[2];
[2]Biol. and Biol. Engin., [3]Div. of Biol. and Biol. Engin., [1]Caltech, Pasadena, CA; [4]California Inst. of Technol. Div. of Biology/Graduate Study In Neurosci., Pasadena, CA; [5]Translational Imaging Ctr., Los Angeles, CA; [6]Janelia Res. Campus / HHMI, Ashburn, VA

Abstract: Sleep is regulated by a homeostatic process and is associated with an increased arousal threshold, but the underlying genetic and neuronal mechanisms remain poorly understood. We performed a genetic screen based on human genome-wide association studies (GWAS) that identified several novel genes whose loss affects sleep. Mutation of one of these genes, *serine/threonine kinase 32a* (*stk32a*), results in increased sleep and impaired sleep homeostasis in zebrafish. We found that *stk32a* regulates sleep by acting downstream of neurotensin signaling and the serotonergic raphe in zebrafish. Phosphoproteomic analysis revealed reduced phosphorylation of neurofilament proteins in *stk32a* mutants, which are co-expressed with *stk32a* in reticulospinal neurons (RSNs) and cranial relay neurons (CRNs) that

promote motor activity, and lateral line neuromasts that detect sensory input such as water flow and vibrations. Ablation of neurofilament-expressing cells or hair cells of lateral line neuromasts phenocopies mutation of *stk32a*. Finally, whole brain calcium imaging revealed that sleep induced by neurotensin stimulation of the serotonergic raphe inhibits motor populations, including RSNs and CRNs, and lateral line neurons that relay sensory information from neuromasts to the brain. These studies thus identify *stk32a* as an evolutionarily conserved sleep regulator that links neuropeptidergic and neuromodulatory systems to homeostatic sleep pressure, which is implemented by suppression of specific sensory and motor systems.

Disclosures: J. Emtage: None. **S. Tran:** None. **M. Lecoeuche:** None. **G. Oikonomou:** None. **A. Andreev:** None. **M.B. Ahrens:** None. **D. Prober:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.22/CC11

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R01HL151853

Title: Microbiota and sleep regulation: the role of hepatic vagus in sleep and fever induced by microbial molecules

Authors: *E. SZENTIRMAI, L. KAPAS;
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Abstract: Emerging research indicates that the intestinal microbiota serves as an important source of sleep-promoting signals. Bacterial metabolites and cell wall components are increasingly recognized as key mediators that link the commensal gut flora to sleep-regulating mechanisms within the brain. These microbial molecules can translocate from the gut lumen into the host's circulation, thereby influencing a range of physiological processes. In previous work, we demonstrated that mimicking the translocation of the bacterial cell wall component lipopolysaccharide (LPS) by direct injection into the portal vein induces both sleep and fever in rats—a response that critically depends on the activity of liver macrophages (Kupffer cells). Building on these findings, the present study was designed to investigate the role of the left vagus nerve, which innervates the liver, in mediating sleep and fever responses triggered by LPS-activated liver macrophages. Male Sprague-Dawley rats were subjected to unilateral cervical vagotomy of the left vagus nerve or sham surgeries (control group) and were instrumented for continuous monitoring of sleep-wake activity, core body temperature, and locomotor activity. On the baseline day, each animal received an intraperitoneal injection of isotonic saline (0.9% NaCl). On the test day, animals were administered 5 µg/kg LPS, intraperitoneally. All treatments were performed 20-30 minutes before the onset of the dark phase in a 12:12 light-dark cycle. Sleep-wake states, body temperature, and motor activity were

recorded for 23.5 hours following each treatment. Data were analyzed using analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) test for post hoc comparisons. LPS administration induced a robust 100% increase in non-rapid-eye movement sleep (NREMS) during the first 6 hours post-treatment (baseline: 85.5 ± 5.1 min vs. LPS: 176.7 ± 8.4 min; $p < 0.001$), accompanied by a marked suppression of motor activity. Body temperature rose by 0.45°C following a 4-hour latency period, sustaining this elevation for 20 hours. Vagotomy attenuated the sleep-modulatory effects of LPS, reducing the NREMS increase to approximately 30% of baseline levels. Furthermore, surgical vagotomy completely abolished the LPS-induced febrile response, demonstrating the critical role of vagal signaling in mediating both the thermoregulatory and somnogenic effects of systemic immune challenge.The results suggest that liver-innervating vagus afferents play a major role in sleep responses induced by LPS translocated into the portal circulation, and they also contribute to LPS-induced febrile responses.

Disclosures: E. Szentirmai: None. **L. Kapas:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.23/CC12

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R01HL151853

Title: Lack of hepatoportal sensitivity to the somnogenic and pyrogenic effects of microbiota-derived peptidoglycan fragments

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Abstract: The intestinal microbiota is a source of sleep-promoting signals. Bacterial metabolites and components of the bacterial cell wall are likely mediators between the intestinal commensal flora and sleep-generating mechanisms in the brain. Microbial molecules translocate from the gut lumen to the host circulation, impacting host physiology. In our previous studies, we demonstrated that the translocation of bacterial cell wall components—such as lipopolysaccharide and lipoteichoic acid—as well as the bacterial metabolite butyrate, act on hepatoportal sensors in the hepatic region to promote sleep. Building on these findings, the present experiments were designed to determine whether fragments of peptidoglycan, another major component of the bacterial cell wall, exert similar sleep-facilitating effects via the hepatoportal mechanism. Male Sprague-Dawley rats were surgically implanted with chronic intravenous catheters in the portal vein and equipped for continuous monitoring of sleep-wake activity, core body temperature, and locomotor activity. We investigated the effects of two

distinct peptidoglycan fragments: muramyl dipeptide (MDP), a component common to both Gram-positive and -negative bacteria, and γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP), a dipeptide fragment specific to the peptidoglycan layer of Gram-negative bacteria. On the baseline day, groups of rats received isotonic NaCl solution, while on the test day, animals were administered MDP or iE-DAP either via direct intraportal vein infusion or by subcutaneous injection, the latter serving to avoid immediate portal circulation absorption of the peptidoglycan fragments. All treatments were administered 20-30 minutes prior to the onset of the dark phase. Sleep-wake states, body temperature, and motor activity were continuously recorded for 23.5 hours following each treatment. Results: Both MDP and iE-DAP induced significant increases in non-rapid eye movement sleep as well as robust febrile responses in the rats. The threshold dose required to elicit these effects for both peptidoglycan fragments was 1,000 µg/kg. Notably, there was no significant difference in the sensitivity of the animals to the sleep- and fever-promoting actions of MDP and iE-DAP, regardless of whether the compounds were administered subcutaneously or directly into the portal vein.Conclusions: These findings indicate that the hepatoportal region does not exhibit uniquely heightened sensitivity to the somnogenic and pyrogenic effects of these peptidoglycan fragments, suggesting that other sites or systemic mechanisms may contribute to their sleep- and temperature-regulating actions.

Disclosures: L. Kapas: None. **E. Szentirmai:** None.

Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.24/CC13

Topic: G.07. Biological Rhythms and Sleep

Support: T32 Sleep & Circadian

Title: Suvorexant'S effect on sleep in female and male c57bl/6 mice during nicotine abstinence

Authors: *S. AKI[1], A. VASSAUX[1], G. MANNINO[1], R. ROWE[1], H. L. MATHEWS[2], J. A. STITZEL[3];
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Abstract: Introduction Nicotine withdrawal disrupts sleep, with females experiencing greater disturbances than males. Poor sleep during withdrawal increases relapse risk. The orexin system regulates both sleep-wake cycles and drug-seeking behavior. It has also been implicated in nicotine addiction behaviors. Therefore, the orexin system could serve as a potential therapeutic target for treating nicotine dependence through multiple mechanisms. Suvorexant, an FDA-approved dual-orexin receptor antagonist for insomnia, improves sleep during opioid withdrawal. Although orexin receptor antagonists have demonstrated that orexin signaling is essential for nicotine addiction in rodents, its role in nicotine withdrawal-induced sleep disruption has not

been assessed. Therefore, the goal of this study is to investigate the effects of suvorexant on sleep in male and female mice during nicotine abstinence. **Methods** To determine sleep-wake behavior, physiological parameters from adult male and female C57BL/6 mice were recorded using a non-invasive piezoelectric cage system. Food and water were provided ad libitum. Following a seven-day acclimation period, baseline sleep was recorded for 48 hours. Nicotine treatment was initiated via osmotic mini-pumps (25 mg/kg/day for 12 days), implanted at ZT0 on Day 1 and terminated at ZT23 on Day 12 to initiate nicotine abstinence. Mice received either suvorexant (30 mg/kg) or a vehicle control (0.5% methylcellulose solution) via oral gavage at ZT0 on Abstinence Days 1 (immediately after mini-pump removal) and 2 (AD1, AD2). **Results** On AD2, female mice treated with suvorexant exhibited a significant increase in total sleep within the first three hours post-gavage, restoring sleep to baseline levels. In contrast, vehicle-treated controls displayed persistent sleep disturbances. Although males did not experience an increase in sleep within the first three hours post oral gavage, they did show an increase in sleep percentage, compared to vehicle controls, at ZT10 on AD2. **Conclusions** These findings provide initial evidence that suvorexant can improve sleep in female and male mice undergoing nicotine abstinence. Given the strong link between sleep disturbances and relapse, further research is warranted to evaluate suvorexant's potential as a therapeutic intervention for nicotine withdrawal-induced sleep disruptions. Targeting the orexin system may provide a novel approach to addressing both sleep and addiction-related challenges during nicotine cessation.

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Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.25/CC14

Topic: G.07. Biological Rhythms and Sleep

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Title: The Dreamy gene, which is identified by forward genetics screening, regulates quantity and quality of REM sleep

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Abstract: Rapid eye-movement (REM) sleep is characterized by the loss of antigravity muscle tone, vivid dreaming, and a wake-like desynchronized electroencephalogram (EEG). While optogenetic and chemogenetic approaches have delineated neuronal circuits triggering REM sleep, the molecular and cellular mechanisms remain largely unknown. To address this, we have performed a large-scale, phenotype-driven forward genetic screening in mice and established a novel pedigree, *Dreamy* (*Dmy*) with markedly increased REM sleep. Linkage analysis and whole-exome sequencing pinpointed a nonsense mutation in the *Dreamy* gene which is causally responsible for the *Dmy* phenotype. Genome-edited mice carrying the *Dmy*-mimic point mutation in the *Dreamy* gene, recapitulated the increase in total REM sleep time with elevated EEG theta power during REM sleep. To dissect functional role of Dreamy, we used AAV-based genetic manipulations of the *Dreamy* gene. Brain-wide AAV-driven knockdown of the *Dreamy* gene increased total REM sleep time with higher EEG theta power during REM sleep. By contrast, local knockdown of the *Dreamy* gene within the hippocampus, an essential generator of EEG theta rhythm, enhanced EEG theta power during REM sleep without altering REM sleep amount. To further identify downstream molecular pathways, we performed single-nucleus RNA sequencing (snRNA-seq) on the hippocampal tissues collected from the AAV-mediated Dreamy knockdown mice. snRNA-seq identified differentially expressed genes in response to the silencing of the *Dreamy* gene among the neuronal subclusters, highlighting potential molecular pathways controlling EEG theta oscillation in REM sleep.Collectively, our results demonstrate that the Dreamy gene regulates both quantity and quality of REM sleep and provides novel insights into unexplored molecular mechanisms regulating REM sleep.

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Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.26/CC15

Topic: G.07. Biological Rhythms and Sleep

Support: Wu Tsai Human Performance Alliance
Joe and Clara Tsai Foundation
George E. Hewitt Foundation for Medical Research
Salk Institute Innovation Grant

Title: Spatial transcriptomics of the energy deficient mouse brain

Authors: *L. VAN ROSMALEN, H. CALLIGARO, V. ROTHENBERG, R. RAMASAMY, S. PANDA;
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Abstract: Relative Energy Deficiency in Sport (REDs) is a syndrome observed in athletes with insufficient energy intake to meet their energy expenditure demands. This results in systemic neuroendocrine, metabolic and behavioral abnormalities. Clinical studies estimate that over 40% of professional athletes suffer from REDs, and we suspect that subclinical forms also affect recreational exercisers who combine calorie restriction with high training loads. To assess the molecular changes in the brain in response to energy deficiency, we implemented the 'work-for-food' paradigm in mice, in which food reward size is determined by wheel-running activity. Using this model, energy-deficient mice show high activity levels and gradually reduced food intake, which results in weight loss and altered rest-activity patterns. We previously showed by performing bulk RNA-seq that energy deficiency dramatically impacts both basal and circadian transcriptomes of 17 different mouse brain regions. Overall, we found distinct gene expression signatures in different hypothalamic, thalamic, and cortical structures. In this study, we performed spatial transcriptomics on coronal and sagittal female and male mouse brain sections to resolve cell-type specific and region-specific transcriptional responses to energy deficiency. Across all 12 samples, we detected 473 million high-quality transcripts from 1.68 million cells and annotated 77 different cell types. The habenula, an evolutionary conserved thalamic brain region involved in depression and the reward system, was largely affected by energy deficiency. Interestingly, within the habenula, most changes were observed in cell types that are in brain barriers and involved in the exchange of molecules into and out of the brain (e.g., ependymal cells, vascular leptomeningeal cells (VLMC's) and choroid plexus). Our findings indicate that the habenula plays a potential role in sensing the animal's metabolic status and regulating downstream adaptive changes in behavior, and may provide important implications for metabolic health, sleep, and performance.

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Poster

PSTR402: Sleep Regulation: Molecular, Cellular, and Pharmacological Aspects

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR402.27/DD1

Topic: G.07. Biological Rhythms and Sleep

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Title: Identification of the key diffusible output factor that regulates circadian behavioral rhythms

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Abstract: In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus plays an important role in regulating circadian rhythms in physiology and behavior. Circadian information from the SCN is output to various brain regions, which in turn regulate physiological functions such as sleep and wakefulness. The SCN was identified as the central circadian clock, and the mechanisms underlying outputs of the SCN in the circadian system have been studied. Although previous studies have highlighted the importance of diffusible factors in regulating circadian behavioral rhythms, it still remains unclear which specific diffusible factors are critical.To identify the key diffusible factor(s) that regulates circadian behavioral rhythms, we performed the isolation of the SCN (iSCN) using a micro-knife. This procedure truncates neuronal axons extending from the SCN to external regions. Importantly, iSCN mice exhibited circadian behavioral rhythms even under constant darkness (DD), indicating the involvement of diffusible factors from the SCN that regulate circadian behavioral rhythms.We then focused on these diffusible factors. Brain-derived neurotrophic factor (BDNF) is known as a candidate molecule that outputs circadian behavioral rhythms. To investigate the role of BDNF in circadian behavioral rhythms, we generated BDNF conditional knockout (cKO) mice by crossing Vgat-cre mice with Bdnf-floxed mice. BDNF cKO (Vgat-cre$^{+/-}$; Bdnf$^{fl/fl}$) mice exhibited robust circadian behavioral rhythms, similar to the control (Vgat-cre$^{-/-}$; Bdnf$^{fl/fl}$) mice under light-dark and DD. We next performed iSCN surgery on BDNF cKO mice to investigate the possibility that circadian behavioral rhythms were maintained by neural connections of the SCN. We found that BDNF cKO mice with iSCN failed to exhibit circadian behavioral rhythms under DD. Moreover, when adeno-associated virus carrying BDNF was injected into the SCN, circadian behavioral rhythms were restored in BDNF cKO mice with iSCN. These results indicate that BDNF is a key diffusible factor for regulating circadian behavioral rhythms.

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Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.01/DD2

Topic: G.07. Biological Rhythms and Sleep

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McKnight Scholar Award
Pew Biomedical Scholar Award

Title: Cortex-specific inversion of visual responses during sleep

Authors: *N. G. CICERO[1], M. KLIMOVA[2], L. VINKE[3], S. LING[4], L. D. LEWIS[5];
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Abstract: During sleep, we functionally disengage from our external environment and our brains curb sensory information from entering our conscious awareness. Most mammals, including humans, sleep with their eyes closed; however, light still penetrates the eyelid and can drive robust responses in the early visual system. Although the relay of sensory information is thought to be gated by the thalamus during sleep, sensory information can still reach the cortex. To elucidate how visual inputs are modulated at each stage of thalamic and cortical processing during sleep, we used simultaneous electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) while presenting luminance-modulated visual stimuli to sleeping humans (n=15). We discovered that while participants were asleep (stages N1 and N2 of NREM sleep), responses to light remained intact in the lateral geniculate nucleus. However, stimulus-evoked responses in visual cortex were profoundly suppressed, exhibiting an inverted pattern in which the degree of suppression scaled with stimulus intensity. Across the entire brain, light only evoked responses within the lateral geniculate nucleus and visual cortex during sleep, whereas other cortical and subcortical regions showed no response. This spatial pattern demonstrated consistent visual-evoked negative responses in sleep, and this focal pattern could not be explained by a global arousal response nor vascular confounds. Rather, these findings suggest a cortical disconnection mechanism whereby inhibitory circuits regulate stimulus-driven suppression in visual cortex, facilitating sensory isolation during sleep.

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Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.02/DD3

Topic: G.07. Biological Rhythms and Sleep

Support: UK Dementia Research Institute
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Title: Falling asleep displays a predictable bifurcation dynamic

Authors: *J. LI[1], A. ILINA[1], R. PEACH[4], T. WEI[1], V. JARAMILLO[5,6], E. RHODES[7,1], I. R. VIOLANTE[2,8], M. BARAHONA[1], D.-J. DIJK[9], N. GROSSMAN[3];
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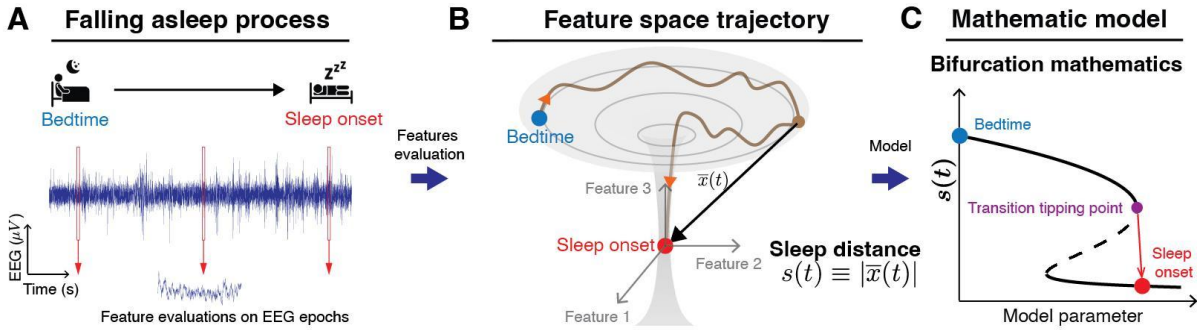
Abstract: Sleep is a fundamental part of our lives, yet how our brain falls asleep remains one of the most enduring mysteries of neuroscience. Within this work, we report a new conceptual framework to analyse and model this phenomenon. The framework represents the changes in brain EEG activity during the transition into sleep as a trajectory in a normalised feature space. We use the framework to show that the brain's falling asleep displays fold catastrophe bifurcation dynamics with a distinct tipping point preceded by a critical slowing down. We validate the bifurcation dynamics in two independent datasets, including more than a thousand human participants. Finally, we demonstrate the framework's utility by predicting individuals' progression into sleep on the fly with seconds temporal resolution and over 0.95 average accuracy.



Disclosures: J. Li: None. **A. Ilina:** None. **R. Peach:** None. **T. Wei:** None. **V. Jaramillo:** None. **E. Rhodes:** None. **I.R. Violante:** None. **M. Barahona:** None. **D. Dijk:** None. **N. Grossman:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

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Program #/Poster #: PSTR403.03/DD4

Topic: G.07. Biological Rhythms and Sleep

Support: Smith Family Foundation, Program for Excellence in Biomedical Research
National Science Foundation, Graduate Research Fellowship Program

Title: Evaluating kinetic modeling and multimodal imaging of endogenous dopamine dynamics during naturalistic sleep-wake transitions in humans using simultaneous PET-EEG-fMRI

Authors: *H. P. FISHER[1,4], I. BASS[5], I. TAWYER[5], N. FULTZ[1], B. SETZER[2,4], S. WILLIAMS[3,4], S. ANAKWE[1,4], B. TAN[4], J. MANDEVILLE[5], C. Y. SANDER[5], L. D. LEWIS[4,5];
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Abstract: Although dopamine is a key neuromodulator regulating arousal, its role in sleep-wake transitions is still not well understood. We aimed to investigate endogenous dopamine changes across sleep-wake states in humans using dynamic [^{11}C]raclopride positron emission tomography (PET) imaging. However, interpreting PET data during variable sleep periods poses methodological challenges because timing and magnitude of neurotransmitter release are uncertain. We therefore assessed how variable timing in dopamine dynamics affect kinetic modeling accuracy, ultimately aiming to investigate dopamine dynamics during arousal state changes. We collected simultaneous PET, electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) data during an afternoon nap opportunity in 15 healthy volunteers. After observing variability in sleep timing, we designed forward simulations to quantify modeling bias introduced by different sleep-onset and duration timing. We employed a two-tissue compartment model to simulate [11C]raclopride time-activity curves with changes in binding due to sleep with variable onset timing, duration, and shapes. Both simulated and experimental data were fit with the multilinear reference tissue model, augmented with a time-varying binding term. Simulations revealed that changes in dopamine occurring late in the experiment (>30 minutes after radiotracer injection) resulted in a negative bias in the estimated change in binding potential. This relationship was consistent across effect sizes and valence of simulated changes. These timing effects were similar across transient and sustained parameter changes at later onset times, but diverged when parameter changes occurred earlier than forty minutes. Kinetic modeling of the experimental PET data revealed increases in [^{11}C]raclopride binding potential aligned to sleep onset. These results were not explained by the trends in modeling bias from our simulations, suggesting a decrease in endogenous dopamine dynamics detectable in human sleep. Our study highlights both the promise and challenges of dynamic PET for investigating dopamine during sleep. Preliminary results of increased binding potential suggest greater dopamine receptor availability, and thus reduced dopamine release, in the striatum during sleep. Rigorous evaluation of timing-related modeling biases is essential for interpreting PET data acquired under naturalistic, endogenous challenges. Additional measurements, such as with EEG and fMRI are essential to guide the kinetic modeling of variable experimental paradigms and to study subtle neurotransmitter dynamics during sleep.

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Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.04/DD5

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Project # 1ZIANS003144-11
NIH Clinical Trials # NCT04095026

Title: Examining hierarchical nesting of slow oscillations, spindles, ripples, and their underlying spiking activity in the human anterior temporal lobe during NREM sleep

Authors: *R. A. VOLKMAN[1], J. I. CHAPETON[2], S. K. INATI[1], K. A. ZAGHLOUL[3]; [1]NINDS, NIH, Bethesda, MD; [2]NINDS, NIH, Natl. Inst. of Neurolog. Disorders & Stroke (NINDS), Bethesda, MD; [3]NINDS, Bethesda, MD

Abstract: Memory consolidation is thought to involve the processes of Synaptic and Systems Consolidation. Synaptic Consolidation involves the refinement of synaptic connections between neuronal cells on the smaller scale of minutes to hours. Critical to this is the co-firing of neurons in short duration (~100 ms) referred to as spike-timing-dependent plasticity (STDP). Systems Consolidation involves memories undergoing spatial reorganization and redistribution between the hippocampus and cortical networks over longer periods of time. Prior work has established the role slow oscillations (SOs), spindles, and ripples play during NREM sleep in these consolidation processes. Communication between the hippocampus and cortical regions is facilitated through hierarchical nesting of hippocampal ripples in cortical SOs and spindles. Additionally, these hierarchical nested events show progressively narrowing underlying co-firing of hippocampal neurons on the temporal scale necessary for STDP. These temporal and spatial relationships have been suggested to exist in a cortical-hippocampal-cortical loop. While the cortical to hippocampal relationship reinstates prior "tagged" memories, there is a return signal yet to be well established in the human literature from the hippocampus to the cortex that drives cortical memory reinstatement. Using 24-hour intracranial EEG recordings and micro-electrode UTAH array single unit recordings in the Anterior Temporal Lobe (ATL) in five patients with focal refractory epilepsy, we hypothesized that single units in the ATL would uniquely modulate their activity when nested within cortical SOs, spindles, and cortical and hippocampal ripples due to the unique role these nested events are thought to play in consolidation compared to isolated events alone. Here we report three results. Firstly, throughout a twelve-hour overnight duration, periods of NREM sleep show higher underlying synchrony (temporal alignment of neuronal firing) compared to wake. Secondly, we observe inter- and intra-regional nesting of these events such that Medial Temporal Lobe (MTL) ripples nest within cortical ATL SOs and spindles, as well as intra-ATL nesting of all three concurring events at rates higher than chance. Finally, we found that the underlying spiking sequence is more similar across nested events compared to non-nested events. These findings provide additional evidence of the dynamics between the hippocampus and cortical regions during NREM sleep for memory consolidation and the mechanisms through which systems and synaptic consolidation involve the ATL.

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Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

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Topic: G.07. Biological Rhythms and Sleep

Support: Stanford Graduate Fellowship in Science & Engineering
Wu Tsai Human Performance Alliance at Stanford University
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Title: Simultaneous EEG-EGG recordings reveal dynamic stomach-brain coupling across NREM sleep

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Abstract: Microarousals (MAs) are brief cortical and autonomic events characterized by transient fluctuations in sleep state. Although they have been traditionally viewed as sleep disruptions, recent evidence suggests that MAs can support sleep-dependent cognitive functions. The locus coeruleus-norepinephrine (LC-NE) system, a neuromodulatory network critical for arousal and memory consolidation, is thought to mediate these MA processes through coordinated infraslow oscillations (ISOs) in the brain and vagally innervated peripheral systems (e.g., heart, blood pressure) during non-rapid eye movement (NREM) sleep. The extent to which LC-NE-linked MAs influence other peripheral organs with high vagal innervation, such as the stomach, during sleep remains unclear. We investigated cortical-autonomic interactions in 60 healthy adults (43 female; 23.2 ± 2.82 years) using overnight 64-channel EEG, ECG, and electrogastrography (EGG) to measure gastric rhythm across three nights. Participants had regular sleep schedules, were medication-free, and underwent memory consolidation tasks. Sleep scoring, artifact removal, and spindle and slow wave oscillation (SWO) detection were performed with validated methods (USleep, High-Density Sleep Cleaner, YASA respectively). Spectral power time series were extracted from the EEG sigma band (9-16 Hz) and EGG gastric band (0.03-0.07 Hz) via multitaper analysis. In a preliminary cohort, we observed a statistically significant prominent 0.02 Hz peak in EEG sigma power, with the highest average ISO power (< 0.05 Hz) localized to occipital regions, and EGG gastric power during NREM sleep. Cross-correlation analyses revealed a significant association between EEG sigma and EGG power (r_{max} = 0.249 ± 0.086; lag = -0.2 s; n = 10). Phase-locking of EGG signals to spindles coupled with SWOs modulated gastric phase more strongly (mean resultant vector length, R = 0.10 ± 0.04, n = 10) than uncoupled spindles (R = 0.05 ± 0.04; p = 0.02, Wilcoxon signed-rank test). We further determined that the occipital EEG-based arousal index, commonly used to assess instantaneous arousal levels, is also strongly correlated with EGG power fluctuations overnight (r_{max} = 0.364 ± 0.079, n = 8). These findings provide the first evidence of coupled cortical-gastric activity during sleep, suggesting that MAs support bidirectional gut-brain communication. By implicating LC-

NE-linked arousal mechanisms in autonomic coordination, this work identifies the gastric rhythm as a novel non-invasive marker for cortical dynamics relevant to sleep, memory, and autonomic regulation.

Disclosures: A.A. Rao: None. **M. Dresler:** None. **S.F. Schoch:** None. **T.P. Coleman:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.06/DD7

Topic: G.07. Biological Rhythms and Sleep

Support: Corundum Convergence Institute (CCI) grant

Title: Closed-loop auditory modulation during sleep to enhance cerebrospinal fluid flow in older adults

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Abstract: Cerebrospinal Fluid (CSF) circulation is a critical process in maintaining brain health, as CSF maintains homeostasis and clears waste from the brain, and CSF disruption is associated with cognitive decline and neurodegenerative disorders such as Alzheimer's disease. CSF flow pulsations are largest during sleep, and are locked to neural slow waves detected in the EEG. Prior studies have increased EEG slow waves during sleep using closed-loop delivery of auditory stimulation. Here, we examined whether specific timing of auditory stimuli during sleep can drive large-scale CSF flow, aiming to optimize an algorithm for inducing CSF flow that is effective in older adults. We first examined a dataset of n=15 young adults (age 18-60 years) who received auditory stimulation during sleep, while recording simultaneous EEG-fMRI. This dataset provided high temporal resolution from EEG and functional imaging from Blood Oxygenation Level Dependent (BOLD) signals, and CSF flow measures in the fourth ventricle. These data had previously indicated that auditory stimuli, phase-locked to ongoing spontaneous electrical activity (EEG slow waves) during sleep, can drive measurable changes in CSF flow. Here, we aimed to determine which EEG features were predictive of the greatest CSF flow changes. We trained an XGboost decoder on auditory stimuli data from younger subjects to predict induced CSF flow,. Our analysis demonstrated that two EEG features significantly predicted the stimulus's effect on CSF dynamics: slow wave (0.5-2 Hz) phase at stimulus onset, and pre-stimulus EEG sleep spindles (11-15 Hz). Building on these findings, we next developed a Closed-Loop Auditory Modulation of Sleep (CLAMS) system designed for older adults to non-invasively enhance CSF flow. The CLAMS system, using an EEG-fMRI-informed approach, acquires scalp EEG signals, applies real-time filtering and artifact correction, and uses a recurrent neural network to predict the phase of ongoing slow waves. Based on this prediction, CLAMS delivers auditory stimuli at optimal timings, as guided by the XGBoost decoder, to

maximize slow wave elicitation based on endogenous neural activity, and drive CSF flow. We explored whether this algorithm elicited distinct effects in older adult participants (age 60-80 years), as compared to the prior dataset in young adults. This work provides insight into neurophysiology drivers of CSF dynamics during sleep, and explores a potential strategy to modulate CSF flow in vulnerable populations.

Disclosures: X. Zeng: None. **J. Levitt:** None. **L.D. Lewis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); L.D.L. is an inventor on a patent application for an MRI method for measuring CSF flow..

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.07/DD8

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant NIGMS R01 GM109086
NIH Grant NIDCD R01 DC004290

Title: Investigation of sleep stage-specific changes in human forebrain single neuron activity

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Abstract: Introduction: Consciousness during rapid eye movement (REM) sleep is unconstrained by connection to the environment, while deep non-REM sleep is characterized by dramatically reduced probability of consciousness. These differences from typical waking consciousness associate with regionally heterogeneous changes in periodic and aperiodic components of the local field potential (LFP) and altered connectivity in the cortico-thalamic network. Metabolic changes such as reduced glucose metabolism during non-REM sleep are observed as well. These changes might be expected to result in altered neuronal single unit activity (SUA), for example reduced firing during non-REM sleep, but findings in preclinical studies are mixed and there has been no systematic study in the human brain.
Methods: To address this knowledge gap, we investigated changes in SUA during overnight sleep in 5 adults (2 male) neurosurgical patients undergoing intracranial EEG monitoring to identify potentially resectable seizure foci. SUA was recorded using Behnke-Fried microwire bundles that extend beyond the tip of the clinical recording electrodes. Overnight sleep data were staged using standard polysomnography. Single neurons were isolated offline and firing rates were examined during 20 - 120 min epochs of wake, REM, NREM sleep (N1, N2, N3). Recording sites were identified using CT- and MRI-guided anatomical reconstructions.
Results: We isolated 357 units in 6 different cortical regions (cingulate cortex, amygdala,

hippocampus, posterior and anterior insula, parahippocampal gyrus). Individual units tracked sleep stage transitions to a variable extent, with subtle changes in firing rate overall. Across all isolated neurons, firing rates were 7.04 Hz (IQR: 6.52) during wake, 5.77 Hz (IQR: 5.85) during N1, 5.61 Hz (IQR: 6.45) during N2, 5.28 Hz (IQR: 6.75) during N3, and 7.01 Hz (IQR: 7.96) during REM. ANOVA indicated an overall effect of sleep stage on firing rate, with significant differences between N3 and wake and between N3 and REM. Units in insula showed the most dramatic differences between REM and wake.
Discussion: These data suggest that on average firing rate decreases during deep NREM sleep, but the changes are modest (~25% for N3 compared to wake) and exhibit some regional heterogeneity. Macroscopic electrophysiological changes (i.e., in the LFP) associated with sleep stages may be reflected in altered temporal firing patterns in addition to changes in firing rate.

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Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.08/DD9

Topic: G.07. Biological Rhythms and Sleep

Title: The impact of sleep quality and resting-functional connectivity

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Abstract: Sleep quality is known to influence brain function, yet the impact of normal day-to-day sleep variation on resting-state functional connectivity (rsfMRI) remains underexplored. This study investigated whether everyday fluctuations in sleep quality affect connectivity within and between the default mode network (DMN) and salience network (SN). Fourteen healthy female subjects participated in two sets of tests that included sleep assessments and MRI scans across two consecutive days. Sleep quality was objectively measured using a portable EEG monitor in the participant's home environment. Prior sleep quality affected connectivity between the DMN and SN, and within the SN. The wake after sleep onset (WASO) index was found to have the strongest relationship with the connectivity between the DMN and SN. The SpD index was also significantly related to the connectivity within the SN. Subjects that had large changes in connectivity tended to have substantial changes in WASO between the two measurements. In contrast, even though there may have been a substantial change in connectivity for a particular subject, the accompanying change in SpD was relatively small. The results suggest that the WASO index is correlated to the DMN-SN interconnectivity, with the observed alteration in

connectivity potentially arising from day-to-day variations in the index. Connectivity associated with the SpD index was observed within the SN, and in contrast to the WASO-related findings, these changes are more likely to reflect individual traits rather than daily fluctuations.

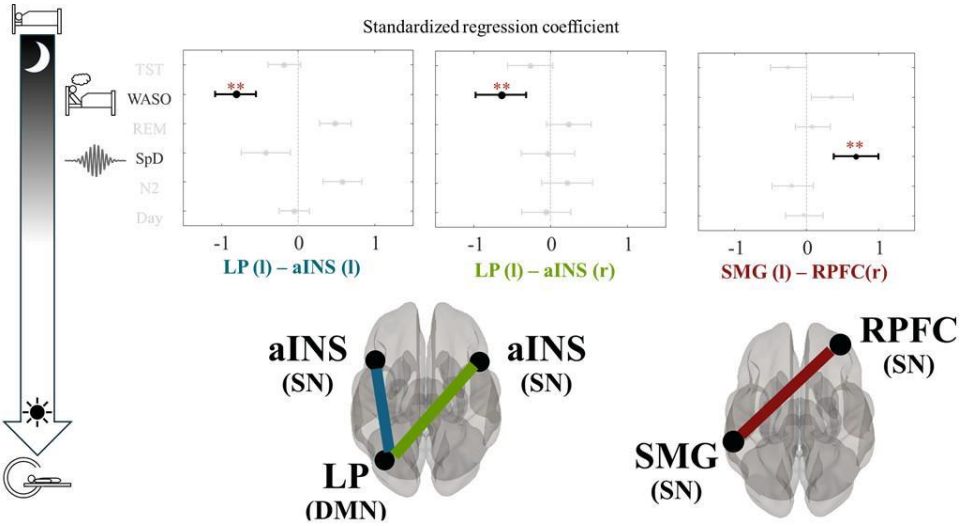

Figure1. Standardized regression coefficients and the most influential independent sleep index (**). Sleep assessment and MRI scanning were conducted on 2 consecutive days. The influence of sleep quality on functional connectivity was examined through a mixed-effects model analysis.

Disclosures: Y. Sahara: None. **M. Yamada:** None. **Y. Hirano:** None. **D. Matsuyoshi:** None. **H. Nishimura:** None. **Y. Aizawa:** None. **N. Yahata:** None. **J. kershaw:** None. **E. Shimizu:** None. **T. Obata:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

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Topic: G.07. Biological Rhythms and Sleep

Support: French Society of Research and Sleep Medecine (SFRMS)
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Title: Inferring alpha-synuclein pathology from emotional valence and 3D kinematics in varied phenotypes of REM sleep behavior disoders

Authors: *S. BOUDEN[1], R. DEBS[2], A. MAZEL[2], A. MARQUE[1], I. BAHAZ LEBBAD[3], C. BOUCLY, Jr[2], M. RAFIQ[2], M. FABBRI[2], A. PAVY-LE TRAON[2], E. BARBEAU[1], J. CUROT[1]; [1]Brain Res. and Cognition Ctr. (CerCo), CNRS, UMR5549, France, Toulouse, France; [2]Dept. of

Neurology, Toulouse Univ. Hospital, France, Toulouse, France; [3]Univ. of Toulouse, Toulouse, France

Abstract: REM Sleep Behavior Disorder (RBD) is an early predictor of alpha-synucleinopathies (SYN), including Parkinson's Disease (PD), Multiple System Atrophy (MSA), and Dementia with Lewy Bodies (DLB), with over 90% of isolated RBD (iRBD) cases converting to SYN within 14 years. RBD can also occur in isolation or secondary to conditions like narcolepsy and Autoimmune Encephalitis (AIE). Despite distinct profiles, limited data differentiate specific RBD features among these diseases. This study highlights RBD heterogeneity by comparing emotional valence and motor kinematic profiles across Narcolepsy, SYN, AIE, and iRBD. We applied a novel classification to assess emotional valence and movement types across 102 RBD patients undergoing polysomnography. A general linear mixed model (GLMM) with Holm correction identified distinctive RBD profiles. Additionally, we analyzed video-polysomnography segments from 38 patients using a convolutional neural network (DeepLabCut) for 2D body keypoint estimation and MiDaS for 3D kinematic tracking. Kinematic measures were statistically compared across diagnostic groups using Kruskal-Wallis and Dunn's post-hoc tests. Unsupervised clustering identified natural groupings based on movement profiles. Results showed more violent movements in SYN-RBD than in narcolepsy ($p=0.04$). Narcolepsy-RBD exhibited more positive emotional valence, while negative emotions prevailed in SYN, AIE, and iRBD (all $p<0.001$). Quantitative analysis confirmed distinct motor profiles, with SYN patients showing greater movement amplitude and velocity, particularly in the right knee (mean amplitude = 308 mm/s in SYN vs 176 mm/s in iRBD, $p=0.03$). AIE patients demonstrated the lowest amplitudes (right knee mean amplitude = 71 mm/s). A graded pattern of motor activity emerged: SYN > iRBD > narcolepsy, indicating varying motor disinhibition levels. Clustering analyses grouped iRBD and SYN cases together, suggesting shared motor patterns, while AIE and narcolepsy reflected lower motor activity. These findings indicate a greater loss of REM atonia in SYN, aligning with a severe RBD phenotype. This observation contrasts with the wake phenotype in each pathology. Identifying these motor biomarkers is crucial for predicting phenoconversion from iRBD to SYN. This pioneering study demonstrates RBD heterogeneity and underscores the importance of integrating qualitative and quantitative biomarkers. Identifying these motor biomarkers is crucial for predicting phenoconversion from iRBD to SYN. We are isolating advanced polygraphic biomarkers from EEG, EOG, ECG, and EMG data to develop an innovative multimodal classification of RBD types.

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Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.10/DD11

Topic: G.07. Biological Rhythms and Sleep

Support: DFG SFB 1280 316803389

Title: Functional connectivity during deep sleep varies by APOE genotype

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Abstract: Despite several studies showing that functional connectivity dynamically changes across sleep stages, it remains unclear how genetic risk factors for Alzheimer's disease (AD), such as carrying the APOE ε4 allele, influence connectivity patterns within each stage of sleep,

as well as how these connectivity changes affect later recognition memory. In this study, we tested the hypothesis that ε4 carriers would show prominent differences in brain connectivity patterns in specific sleep stages relative to either non-carriers (ε3/ε3) or ε2 carriers, and that these differences would be associated with poorer recognition memory performance in the carrier group. Seventy-nine participants were scanned using fMRI and genotyped for APOE. During encoding, participants viewed images of either faces or buildings and judged whether they liked/disliked each face/building. After encoding, they slept inside the scanner for one hour, while whole-brain fMRI was recorded and sleep monitored with EEG. Next, an fMRI localiser was run to identify face- and place-specific brain areas which were used as regions of interest (ROI) in subsequent analyses. In the final phase, participants decided whether faces/buildings had been shown during encoding using a 1 (old) - 6 (new) scale. Behaviourally, there were no differences in memory performance among the three APOE groups. However, our fMRI results showed that, relative to non-carriers, the ε4 group generally exhibited lower connectivity between several ROIs (e.g., entorhinal-parahippocampal cortices) during deeper sleep stages (particularly N3), whereas wake connectivity did not differ. Interestingly, ε2 carriers showed greater deep-sleep functional connectivity between most pairs of ROIs relative to the other groups. Whole-brain connectivity analysis also indicated stronger functional connectivity between the parahippocampal cortex and frontal regions in ε2 carriers relative to both ε4 carriers and non-carriers. Our results suggest a local circuit that could be impaired in ε4 carriers during deep sleep and highlight functional connectivity differences in ε2 carriers that may support compensatory mechanisms underlying their lower AD risk.

Disclosures: C. Gomes: None. **L. Deuker:** None. **N. Axmacher:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.11/DD12

Topic: G.07. Biological Rhythms and Sleep

Title: Sleep quality in the adult population of western Mexico

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Abstract: Sleep is a decreased in consciousness that prevents us from reacting to external stimuli. It is a physiological activity that balances a person's physical and mental well-being. Recent studies suggest that exercising, avoiding stimulant beverages, avoiding the use of electronic devices, and maintaining a relaxing routine such as meditation several hours before bedtime can help improve sleep quality. The objective of this research is to determine the sleep quality of the population of western Mexico. The Pittsburgh Sleep Quality Index (PSQI) was

applied. The sample size was determined using a projection of the total population of the state of Jalisco by 2025, which is 8,931,497 people. Because there are no current reports on sleep quality statistics in Mexico, the sample size was determined based on a 50/50 ratio, using Epidat software (version 4.2, 2016). A sample of 429 adults was collected, mean age of 32.37 ± 12.6 years. The sample consisted of 279 women and 150 men, all residents of western Mexico. The majority of the participants were university students and healthcare professionals (physicians and psychologists). The most common bedtime was 11:00 pm and the most common wake-up time was 7:00 am, spending 8 hours in bed, referring a total of 6 hours of sleep, and their habitual sleep efficiency score was 75%. The evaluation of all items on the PSQI averaged a score of 10, which indicates bad sleep quality. Based on the data collected, we concluded that participants spent an adequate amount of time in bed, but took a long time to fall asleep, and therefore tended to have poor sleep quality.This study was conducted in accordance with the Mexican Official Standard NOM-012-SSA3-2012.

Disclosures: J. Guillen Ruiz: None. **L. Becerra-Solano:** None. **M. Macías-Carballo:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.12/DD13

Topic: G.07. Biological Rhythms and Sleep

Title: Identifying neural networks implicated in narcolepsy through lesion network mapping

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[1]Brigham Young Univ., Provo, UT; [2]Harvard Med. Sch., Boston, MA

Abstract: Narcolepsy is a hypersomnia disorder, characterized by excessive daytime sleepiness and at least one REM-sleep dissociation symptom: cataplexy, hypnagogic/hypnopompic hallucinations, sleep paralysis, short mean sleep latency, or multiple sleep onset REM periods. Previous research using voxel-wise lesion symptom mapping methods has failed to identify a specific neural network responsible for narcolepsy, but has identified regions that could be relevant, including the hypothalamus and thalamus. The primary objective of this study is to locate principal networks involved in lesion-induced narcolepsy. We utilized lesion network mapping, a neuroimaging method that examines connectivity pathways in the brain to localize active sites for symptoms of interest. Through systematic literature review using specific inclusion criteria, we identified relevant case reports for patients presenting with symptoms of narcolepsy (n=22). Lesions principally resulted from encephalitis, tumors, autoimmune disease, inflammation, and neural infection. Lesion network mapping analysis was performed on 22 cases, with a large body of healthy control resting-state scans (n=1000). Network conjunction analysis revealed that 95% of cases were functionally connected to the hypothalamus (21/22). Furthermore, we found that 95% of cases were functionally connected to the parahippocampal gyrus and the portions of the anterior temporal lobe. Our results align with current research,

which demonstrates that sleep disorders have an established relationship with hypothalamic abnormalities. However, our study reveals additional novel regions that may impact sleep disorders. The parahippocampal gyrus seems to be associated with the default-mode network of the brain which has been implicated in sleep disorders previously. However, the relationship between the parahippocampal gyrus and narcolepsy is not well understood. A relationship between the temporal lobe and narcolepsy has not been fully explored either. Further research is required to better understand how these networks interact and the role they play in contributing to narcolepsy. Future study of these active sites will enable clinicians to gain a more comprehensive understanding of narcolepsy, as well as facilitate targeted patient interventions.

Disclosures: C. Spillett: None. **J. Griffin:** None. **M. Ferguson:** None. **J.A. Nielsen:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH grant T32 MH064913
NIH grant R01 NS112252

Title: Identifying if network switching rate may be a brain marker of arousal dependent cognitive changes

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[1]Neurosciences, [2]Vanderbilt, Nashville, TN; [3]Biomed. Engin., [4]Computer Sci., [5]Neurolog. Surgery, Vanderbilt Univ., Nashville, TN; [6]Vanderbilt Memory and Alzheimer's Ctr., [7]Vanderbilt Univ. Med. Ctr., Nashville, TN; [8]Vanderbilt Univ. Med. Ctr., NA, TN; [9]Psychology, UCLA, Los Angeles, CA; [10]Electrical and Computer Engin., Vanderbilt Unversity, Nashville, TN

Abstract: Arousal state fluctuations are typically accompanied by cognitive changes. However, the brain-network interactions that underlie arousal-dependent cognitive changes have not been fully elucidated. One measure that has been associated with cognitive performance is network switching, which quantifies how frequently a brain network transitions between functional network communities. Previously, we found that the network switching rates of the dorsal default mode network (DDMN) and the left central executive network (LCEN) were sensitive to arousal state. However, whether arousal state moderates the relationship between network switching rate and cognitive performance has, to our knowledge, is unknown. Here, we first investigate if the switching rates of the DDMN and LCEN would relate to a cognitive function composite score and if arousal state moderates any significant relationships. In addition to

examining networks, we also tested if arousal state moderates the relationship between global network flexibility (i.e., the average network switching rate across all networks) and certain cognitive measures. This second analysis is motivated by prior literature which has shown that global network flexibility predicts working memory task and relational task accuracy scores. We used the Human Connectome Project 7T dataset, drawing upon 133 subjects who had resting-state fMRI scans, eye-tracking data, and the cognitive measures of interest. Resting-state networks were derived from an established atlas of 14 networks. We closely followed the methods presented in Pedersen et al., 2018 to determine network switching rates. To determine the arousal state of each scan, we used participants' eye-tracking data, classifying a scan as alert if participants had eye closures present for at most 5% of the scan, and as drowsy if they had eye closures between 50-90% of the scan. We used a linear regression model to assess the relationship between network switching rate and cognitive measures. For our first analysis, we found that the switching rate of the LCEN was significantly related to the cognitive function composite score after Bonferroni correction for multiple comparisons ($\beta = 2.09$, $p = .02$), and that arousal state did not moderate this relationship ($\beta = -1.39$, $p = .312$). For our second analysis, we found that arousal state moderated the relationship between global network flexibility and relational task accuracy ($\beta = -5.09$, $p = .01$), but not working memory task accuracy ($\beta = -1.25$, $p = .27$). These findings suggest that global network flexibility may be a brain marker of certain arousal-dependent cognitive changes.

Disclosures: K. Rogge-Obando: None. **H. Pourmotabbed:** None. **S. Wang:** None. **K. Kaur:** None. **Y. Li:** None. **S. Goodale:** None. **C. Martin:** None. **V.L. Morgan:** None. **D.J. Englot:** None. **L.Q. Uddin:** None. **M. Rubinov:** None. **C. Chang:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.14/DD15

Topic: G.07. Biological Rhythms and Sleep

Support: UCLA School of Nursing intramural
NIH Grant NR017435
NIH Grant HL135562

Title: Inspiratory muscle training in obstructive sleep apnea: influences on breathing and neuroinflammation (a pilot study)

Authors: *P. M. MACEY;
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Abstract: Introduction: Obstructive Sleep Apnea (OSA) affects 15% or more of the population and leads to higher morbidity and mortality. Continuous positive airway pressure (CPAP) ameliorates many symptoms, yet blood-pressure (BP) benefits are modest. However, BP is

lowered by a non-invasive intervention known as Inspiratory Muscle Training (IMT). To better understand potential mechanisms, this study investigated the effects of IMT in OSA on respiratory patterns and brain structure reflecting inflammation. Methods: Five participants with OSA (2 females; age range 47-74, mean±std 62±5 years) completed ten weeks of IMT involving daily sessions of 30 inspiratory breaths against a resistance of 65% of maximal inspiratory pressure. Data collected at baseline and follow-up consistent of MRI and physiology recordings. Brain structural changes with IMT were assessed using voxel-based morphometry (gray matter volume, higher with improved tissue health) and diffusion tensor imaging indices (mean diffusivity, lower with higher inflammation). Additionally, respiratory patterns were analyzed to assess the impact of IMT on resting breathing. Results: IMT led to changes in breathing and neural measures. Respiratory patterns showed normalization, with the inspiration-to-expiration ratio aligning with healthy controls post-IMT. Brain imaging revealed increased mean diffusivity in multiple brain areas, with large effect sizes in some regions ($p < 0.05$ in ventral prefrontal, lingual gyrus, temporal regions). Increased gray matter volume appeared in similar regions as well as the right insula and hippocampus. Conclusions: This pilot study demonstrates that a 10-week, high-adherence IMT regimen can both regularise resting breathing and produce macro- and micro-structural brain improvements that imply lower neuroinflammatory burden. These neural and physiological shifts offer a possible substrate for the blood-pressure reductions reported with IMT and support larger-scale trials testing IMT as an adjunct or alternative to CPAP in OSA management.

Disclosures: P.M. Macey: None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

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Topic: G.07. Biological Rhythms and Sleep

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Conte Center on the Neurocircuitry of OCD MH10643
McKnight Foundation Award

Title: Arousal effects on oscillatory dynamics in the non-human primate brain

Authors: *S. ANAND[1], F. SOGUKPINAR[2], I. E. MONOSOV[3];
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Abstract: Arousal states are thought to influence several aspects of cognition and behavior by broadly modulating neural activity. Many studies have observed arousal-related modulations of alpha (~8 to 15 Hz) and gamma (~30 to 50 Hz) power and coherence in local field potentials across relatively small groups of brain regions. However, the global pattern of arousal-related oscillatory modulation in local field potentials is yet to be fully elucidated. We simultaneously recorded local field potentials in numerous cortical and subcortical regions in the primate brain and assessed oscillatory activity and inter-regional coherence associated with arousal state. In high arousal states, we found a uniquely strong and coherent gamma oscillation between the amygdala and basal forebrain. In low arousal rest-like states, a relative increase in coherence at alpha frequencies was present across sampled brain regions, with the notable exception of the medial temporal lobe. We discuss how these patterns of activity may index arousal-related brain states that support the processing of incoming sensory stimuli during high arousal states and memory-related functions during rest.

Disclosures: S. Anand: None. **F. Sogukpinar:** None. **I.E. Monosov:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.16/EE1

Topic: G.07. Biological Rhythms and Sleep

Support: NSF Grant IIS 2342866
NIDCD Grant R01DC019278

Title: Electrophysiological characterization of daytime naps in the marmoset

Authors: J.-M. FELLOUS[1], D. NGUYEN[2], *Y. ZHOU[2];
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Abstract: Sleep is an important part of brain function. The effects of sleep on cognition are well documented in humans and rodents, including its role on improving memory consolidation, achieving synaptic scaling, improving working and executive memory in addition to general restorative effects. Much less is known of daytime naps. Even though naps include short partial interrupted sleep epochs of variable neural rhythmic composition, they have shown to yield benefits similar to that of full night sleep. It is not clear whether the neural mechanisms underlying these effects are in fact the same as for sleep or to what extent they are similar. Animal models of naps are difficult to study. For example, rats are nocturnal and have polyphasic sleep patterns that make their naps difficult to identify or quantify. Marmosets on the other hand are diurnal and present monophasic sleep patterns very similar to that of humans. This study investigated the neurophysiological signatures of naps based on the analysis of local field potentials (LFPs) in marmosets. LFPs were collected from the primary auditory cortex in two chair-trained marmosets using high-density silicon probes. Each recording session started when

the animal's eyes were closed and lasted about 30 minutes. Animals did not perform any tasks and no sensory stimuli were presented. Eye movement and pupil diameter were continuously collected to characterize nap and non-nap epochs. We identified and quantified the occurrence of nap-induced slow waves, cortical sharp waves and spindles, and measured the relative power and phase shifts between inter-laminar LFP signals. These results were compared to those collected in the awake and behaving condition to discover the nap-induced dynamics of brain waves in the primate brain. This pilot study sets the stage for further work aimed at measuring the extent to which nap features reflect past or predict future behavioral performance.

Disclosures: J. Fellous: None. **D. Nguyen:** None. **Y. Zhou:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.17/EE2

Topic: G.07. Biological Rhythms and Sleep

Support: NIH-NINDS Grant NS111019
Royal Academy of Engineering, UK

Title: Real-time regulation of arousal and performance in healthy nonhuman primates using the bidirectional neuromodulation research platform, DyNeuMo-X

Authors: *J. L. BAKER[1], R. TOTH[4], M. ZAMORA[4], A. DELI[5], J. E. FLEMING[6], M. BENJABER[4], J. J. VAN RHEEDE[6], J. RYOU[2], N. D. SCHIFF[1], K. P. PURPURA[3], T. DENISON[4];
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Abstract: Recent advancements in bidirectional neuromodulation systems like closed-loop deep brain stimulation (DBS) and brain-computer interfaces (BCI) hold great promise for enhancing our understanding of the brain and for developing the next-generation of technology to restore lost capacity. Treatment-resistant brain disorders are manifestations of aberrant neuronal activity in widely distributed brain networks. And invasive approaches like DBS and BCI are emerging as promising therapeutic options for most indications. In recent clinical studies, central thalamic DBS (CT-DBS) significantly improved executive attention and restored cognitive function in five moderate-to-severe traumatic brain injury (msTBI) patients (NCT02881151) and helped reverse post-ictal cognitive dysfunction in three temporal lobe epilepsy (TLE) patients (NCT04897776). However, we still lack a clear understanding of how CT-DBS affects both brain areas and broader networks in the injured brain, primarily due to our inability to effectively

monitor and modulate the brain's daily, circadian rhythm of arousal within the anterior forebrain network (AFN). To address key limitations of current DBS systems, we developed and validated a clinical-grade bidirectional neuromodulation system, the DyNeuMo-X, in two healthy behaving non-human primates (NHP). We used pupillometry, behavioral performance measures and real-time analysis of thalamic LFP and prefrontal and premotor ECoG signals to initiate closed-loop CT-DBS episodically, with the goal of restoring and enhancing behavioral performance. In exploratory studies, the DyNeuMo-X was programmed to deliver closed-loop CT-DBS during the night, which significantly modulated oscillations present during sleep in one animal. This nighttime intervention markedly impaired the animal's daytime performance of cognitively demanding tasks that require sustained executive attention over several hours. This result mirrors key aspects of the chronic sequalae experienced by patients with structural brain injuries and a wide range of neurological disorders. The implantable DyNeuMo system is currently in use in several ongoing (NCT03837314, NCT06387914) and recently completed (NCT05197816, NCT05437393) clinical trials in the UK, and the approach developed here validates the use of closed-loop DBS in a large animal model for further preclinical studies. This work supports our long-term goal of developing new systems to modulate the circadian dynamics of arousal regulation withing the AFN, to more effectively treat cognitive dysfunction in patients with structural brain injuries and further etiologies.

Disclosures: J.L. Baker: None. **R. Toth:** None. **M. Zamora:** None. **A. Deli:** None. **J.E. Fleming:** None. **M. Benjaber:** None. **J.J. Van Rheede:** None. **J. Ryou:** None. **N.D. Schiff:** None. **K.P. Purpura:** None. **T. Denison:** None.

Poster

PSTR403: Kinematics of Sleep-Arousal States: Human and Non-Human Primate

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR403.18/EE3

Topic: G.07. Biological Rhythms and Sleep

Support: NWO; project number 1292.19.077

Title: Long-wavelength sensitive photoreceptors mediate sleep suppressing effects of acute artificial light at night in diurnal tree shrews (Tupaia belangeri)

Authors: *K. VITKUTE[1], R. HAVEKES[2], P. MEERLO[2];
[1]Neurobio., [2]Groningen Inst. for Evolutionary Life Sci., Univ. of Groningen, Groningen, Netherlands

Abstract: The expansion of urbanization and the proliferation of artificial light at night (ALAN) have rapidly reshaped nocturnal environments. Together with convenience it has come at a cost on both individual and ecosystem levels, with most conspicuous consequence being disturbance of regular sleep patterns across species. While short-wavelength blue light is particularly disruptive to human sleep, it may not generalize to other mammals, which could experience

distinct vulnerabilities to ALAN. Here, we investigate how ALAN intensity and wavelength influence sleep and behavioral patterns in diurnal tree shrews, semi-arboreal mammals closely related to primates. Eight adult tree shrews (4 males, 4 females) were implanted with custom electrodes to measure brain temperature, brain activity (electroencephalogram, EEG), and muscle tension (electromyogram, EMG). The signals were recorded with miniature wireless dataloggers (Neurologger 2A) under freely moving conditions. Each experiment consisted of 24 hours of baseline recording, 12 hours of exposure to ALAN followed by a 24-48 hour recovery period. Initial experiments comparing dim (3 lux) and moderate (30 lux) white ALAN revealed significant alterations in brain wave intensity, increased sleep fragmentation, and notable reductions in NREM and REM sleep, effects amplified at higher light intensities. Intriguingly, sleep-disrupting effects of low intensity ALAN were predominant in females, but not in males, suggesting sex-specific thresholds in light sensitivity. Tree shrews have highly developed cone-dominated retinas, with two cones sensitive to either short- or long- wavelength light (S-cone and L-cone). To further elucidate which cones are the main contributors to the observed effects of light, animals were exposed to either blue or green ALAN, targeting both cones at intensity matched to that of white ALAN. Green ALAN imposed markedly greater sleep disruptions compared to blue ALAN, though neither equaled the sleep disruption severity of white light. These results indicate the involvement of multiple retinal pathways, with L-cones potentially playing a primary role. Our findings reveal complexity in how different wavelengths drive ALAN-induced sleep loss and warrant further investigation of the immediate and long term consequences of ALAN on mammalian physiology. Understanding these dynamics will be critical for developing informed guidelines to mitigate human-induced environmental pressures, such as light pollution.

Disclosures: K. Vitkute: None. **R. Havekes:** None. **P. Meerlo:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.01/EE4

Topic: G.07. Biological Rhythms and Sleep

Title: Long-term changes in blood pressure and sleep architecture during and after the CoV-19 pandemic

Authors: *D. M. WEINERT[1], H. D. BENNEFELD[2];
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Abstract: This long-term study of a test subject with two mild SARS-CoV-2 infections and four mRNA-based vaccinations utilizes a self-tracking system to evaluate blood pressure and sleep data over a 53-month period. The initial results encompassed daily data from a test subject from December 30, 2020, to April 30, 2024, and have since been augmented with more recent data up

to May 27, 2025. The occurrence of CoV-19 infections was documented in 2021 (Ct=34.42) and 2002 (Ct=27.09, Omicron), both on March 10. The entire period of 4.4 years was reclassified into the following phases: a pre-covid phase (70 days), a post-covid-1 phase (350 days), a post-covid-2 phase (770 days), and a post-covid-3 phase (392 days). Point-of-care rapid test results were negative on the day preceding the infection. Following the positive confirmation of SARS-CoV-2 RNA, the subsequent 14-day period was examined separately. An evaluation of activity data and internal parameters was conducted, encompassing daily steps, body temperature, weight and body mass index, blood pressure, heart rate, VO2max, and pulse wave velocity. The evaluation also incorporated sleep data, including REM, DEEP, and LIGHT sleep, as well as total and restorative sleep. The collected data were subsequently subjected to statistical analysis to identify any deviations over time. Statistically significant alterations in blood pressure and sleep architecture parameters were not identified in the pre-covid phase. In the post-covid-1 phase, systolic blood pressure values increased (P=0.0001). A decline in the duration of LIGHT sleep was observed (P=0.0094), accompanied by an increase in the duration of restorative sleep (P=0.0137) and a rise in heart rate (P=0.0001). Following Omicron infection, a significant increase in systolic and diastolic blood pressure values was observed in the post-covid-2 phase (P=0.0001 each). Regarding sleep parameters, a notable shift towards increased LIGHT sleep (P=0.0056) and concurrent decrease in DEEP and REM sleep (P=0.0001 each) was evident. In the post-covid-3 phase, a significant decrease in systolic (P=0.0036) and diastolic (P=0.0010) blood pressure parameters was observed 2.1 years after the second infection with the Omicron variant of the SARS-CoV-2 virus. As the DEEP phase remains constant, there was an observed increase over time in the REM phase (P=0.0005) and the LIGHT phase (P=0.0003). The findings reveal a persistent effect on blood pressure and sleep metrics, with no requirement for inpatient hospitalization. Moreover, after a period of 2.1 years, a substantial decrease in blood pressure values and an increase in REM phases can be observed, at least in this particular case.

Disclosures: D.M. Weinert: None. **H.D. Bennefeld:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.02/EE5

Topic: G.07. Biological Rhythms and Sleep

Support: NSF CAREER 2237419

Title: Habitual Sleep Prior to Enrollment Predicts Future Stress-Related Measures in Emergency Responders

Authors: E. LARIOS, *I. LERNER;
The Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Sleep, particularly Rapid-eye Movement (REM) sleep, plays a critical role in psychological functioning and stress resilience. While a bidirectional relationship between sleep and stress is well-established, existing research has rarely examined whether pre-existing habitual sleep patterns, as measured by objective physiological measures, predict psychological outcomes months or even years later. Our current ongoing longitudinal study addresses this gap by examining whether objective baseline sleep metrics prospectively predict vulnerability or resilience to psychological distress in emergency responders, including police officers, firefighters, and paramedics. Participants are enrolled at the onset of academy training and monitored over an 18-month period with assessments conducted at four time points: pre-training (T1), post-graduation (T2), and 6- and 12-months into field duty (T3, T4). At T1, participants complete a seven-day mobile sleep assessment, which capture detailed metrics of sleep architecture including duration of time spent in each sleep stage, sleep efficiency, and total sleep time. Validated self-report instruments are administered at each timepoint to evaluate symptoms of acute stress, PTSD, burnout, and protective factors such as compassion satisfaction and resilience. Here, we present interim results from this study. Analysis revealed statistically significant associations between sleep and psychological outcomes across time. Higher baseline REM duration was associated with increased emotional responsiveness to aversive events prior to training and predicted greater compassion satisfaction during the transition into active duty. In addition, baseline sleep efficiency was similarly predictive: individuals with more consolidated sleep exhibited lower levels of burnout and acute stress, fewer sleep complaints, and greater indicators of emotional recovery across later timepoints. Together, these findings suggest that objective sleep characteristics may serve as early markers of stress resilience and vulnerability. REM sleep, in particular, may reflect a capacity for emotional integration, influencing how individuals absorb and respond to trauma over time By identifying individuals who may be predisposed to distress before exposure to occupational trauma, sleep metrics could inform targeted intervention and prevention strategies in high-risk professional settings. Ongoing data collection and trajectory modeling are underway to further illuminate how early sleep profiles shape long-term psychological outcomes in high-risk professions.

Disclosures: E. Larios: None. **I. Lerner:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.03/EE6

Topic: G.07. Biological Rhythms and Sleep

Support: U54MD012393 (Subproject: 5378)
K01DA037819
Florida Department of Health grant 24K09

Title: Sleep Disruption Impacts Brain Regions Related to Substance Use Risk Behaviors

Authors: *K. E. CROOKS[1], P. M. VIERA PEREZ[2], M. T. SUTHERLAND[1];
[2]Cognitive Neurosci., [1]Florida Intl. Univ., Miami, FL

Abstract: Sleep disruption has been associated with changes in brain activity, multiple cognitive alterations, and several neuropsychiatric conditions. These disruptions have been linked to increased risk-taking behaviors and substance use (SU). Understanding how sleep deficiency affects behavior is crucial for developing targeted interventions to mitigate these risks. We conducted coordinate-based meta-analyses to delineate common and distinct brain activity alterations across functional neuroimaging studies assessing the impact of sleep disruption via between-subjects (disorders, *n*=60) and within-subjects designs (deprivation, *n*=35) as well as structural neuroimaging studies assessing the impact of sleep disruption via parasomnias (disruption of sleep cycles, *n*=17) and dyssomnias (disruption of falling or staying asleep, *n*=40). Meta-analytic connectivity models were additionally conducted to delineate regions that functionally connect with the regions of interest, thereby characterizing an extended network. Sleep disruption was associated with convergent brain activity decreases in the dorsomedial and ventrolateral prefrontal cortex (PFC); when focusing only on disorder-related studies, convergent activity decreases were observed in the dorsomedial PFC. Sleep disorders were associated with convergent structural integrity alterations in the thalamus. When focusing only on parasomnia-related studies, convergent structural integrity decreases were observed in the posterior cingulate cortex (PCC). Linking brain alterations with putative behavioral consequences, the ventrolateral PFC and PCC were both associated with behaviors relating to judgment and decision-making, the dorsomedial PFC was associated with behaviors related to negative affect and social processing, and the thalamus was related to both sleep behaviors and performance of tasks. Disrupted neural connectivity linked to sleep disturbances underlie risk-taking and substance use behaviors. Insufficient sleep can exacerbate or prolong these tendencies, emphasizing the importance of addressing sleep issues to reduce risky behaviors. Regions that have been identified will be used as seeds of connectivity analysis for adolescents, a group particularly vulnerable to alterations in the behaviors listed and in sleep disruption.

Disclosures: K.E. Crooks: None. **P.M. Viera Perez:** None. **M.T. Sutherland:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.04/EE7

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant AA028175-01
VA Merit Award I01BX002661
VA Merit Award I01BX006240-0

Title: Serotonergic Mechanisms in the Dorsal Raphe Nucleus Mediate Fentanyl-Induced Wakefulness and Sleep Disruption

Authors: *M. M. THAKKAR, **R. SHARMA**, M. PARIKH;
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Abstract: The dorsal raphe nucleus (DRN), the brain's largest serotonergic nucleus, plays a central role in regulating arousal and S-W states via extensive 5-HT projections and reciprocal connections with arousal centers, including the orexinergic lateral hypothalamus (ORX-LHA). Although serotonin (5-HT) is implicated in the effects of opioids, its specific role in fentanyl-induced sleep disruption remains unexplored. We hypothesized that fentanyl disrupts S-W cycles via 5-HT-dependent mechanisms within the DRN. To test this, adult male C57BL/6J mice (N=5) implanted with EEG/EMG electrodes were used for polysomnographic recordings. At ZT6, saline (10 ml/kg) was systemically administered on Day 1 followed by fentanyl (1.23 mg/kg, i.p.) on day 2 at same time point. After three days, brain serotonin was depleted using systemic *p*-chlorophenylalanine (pCPA; 400 mg/kg, twice daily for three days; N=4). After three days post-pCPA, saline (10 ml/kg) or Fentanyl (1.23 mg/kg, i.p.) was administered as described above. Sleep-wake states were recorded for 6 hours post-saline or fentanyl injection and analyzed in two 3-hour intervals. Our preliminary results suggest that during the first 3 hours post-injection, fentanyl significantly increased wakefulness compared to saline (Saline: 30.4 ± 2.0%, Fentanyl: 94.5 ± 1.1%, $p<0.001$), and reduced both NREM (Saline: 60.5 ± 1.5%, Fentanyl: 3.5 ± 0.9%, $p<0.001$) and REM sleep (Saline: 9.0 ± 1.1%, Fentanyl: 1.9 ± 0.9%, $p<0.01$). Following 5-HT depletion, fentanyl-induced wakefulness was attenuated (85.5 ± 1.6%, $p<0.05$ vs. Fentanyl), with partial restoration of NREM (11.4 ± 2.0%) and REM sleep (3.1 ± 1.1%). During the second 3 hours, fentanyl-treated animals exhibited prolonged wakefulness relative to saline controls (Saline: 31.7 ± 3.5%, Fentanyl: 43.3 ± 5.0%), though less pronounced than during the initial 3 hours. NREM sleep was also decreased (Saline: 59.5 ± 3.6%, Fentanyl: 47.4 ± 6.3%), while REM sleep remained suppressed (Saline: 9.3 ± 1.9%, Fentanyl: 9.3 ± 5.7%). Post-pCPA, fentanyl-induced sleep disruption was again attenuated (Wake: 28.5 ± 1.4%, NREM: 66.4 ± 1.9%, REM: 5.2 ± 0.8%). These results demonstrate that fentanyl-induced wakefulness is mediated by serotonergic mechanisms, implicating the DRN in opioid-related sleep disturbances.

Disclosures: M.M. Thakkar: None. **R. Sharma:** None. **M. Parikh:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.05/EE8

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant UH3 DA050325

Title: Effect of GLP-1 receptor agonist semaglutide on sleep and food/water intake in rats

Authors: *E. G. SHEALY[1], A. HAJNAL[2], P. S. GRIGSON[3], J. FANG[4];
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Abstract: Opioid Use Disorder (OUD) is characterized by cycles of binge, withdrawal and relapse. Sleep disturbances occur following acute and chronic opioid use and contribute to this cycle by increasing craving. Recent work has shown that glucagon like peptide 1 receptor agonists (GLP-1RAs) reduce opioid seeking in rodent models and promote sleep in patients. Our lab found that the GLP-1RA liraglutide, a once daily injection, decreases wake time and increases NREM sleep over a period of 24 hours. However, daily dosing poses a heavy burden for patients in clinic. Semaglutide, another GLP-1RA dosed weekly, has yet to be evaluated for sleep effects. We hypothesize that semaglutide will decrease waking/increase NREM sleep for 48 hours. Further, GLP-1RAs promote expression of pro-opiomelanocortin (POMC), a satiety peptide that can promote sleep through signaling in the Arcuate Nucleus (Arc) of the hypothalamus. Thus, we also predicted an increase in POMC expression in the Arc following treatment with semaglutide. EEG and EMG implantation were performed for 15 adult male drug-naïve Sprague-Dawley rats. Subjects were subcutaneously (s.c.) injected with saline or semaglutide using a within-subjects ABA design and doses previously shown to reduce fentanyl seeking (0.026, 0.056, and 0.078 mg/kg). EEG and EMG were recorded for the 24h period following each injection, with saline or semaglutide injections occurring one hour prior to dark onset; or, in a follow up study, at light onset using a 12:12 light-dark cycle. Along with sleep, food intake, water intake, and body weight were monitored daily as semaglutide is FDA-approved for weight loss. Following the last recording, animals were injected with 0.056 mg/kg semaglutide and sacrificed 2 hours later. The Arc was dissected to assess POMC expression via rt-qPCR. During the dark cycle, similar to liraglutide, all doses of semaglutide significantly decreased food intake, water intake and body weight. Assessment of the effect of semaglutide on sleep and Arc POMC expression is ongoing. If our hypothesis is supported, these preliminary data will inform the mechanisms of GLP-1RAs as candidate OUD therapies. If semaglutide, like liraglutide, promotes sleep, this may render it preferable to liraglutide due to the need for less frequent dosing. This work was supported by NIH Grant UH3 DA050325.

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Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.06/Web Only

Topic: G.07. Biological Rhythms and Sleep

Support: BUAP-CA-288
SECIHTI No. 926368

Title: Early-age alcohol intake produces changes in sleep-wake cycle in adult High- and Low-yawning rats

Authors: *A. FIERRO-ROJAS[1], C. CORTES[2], J. R. EGUIBAR, Sr.[3];
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Abstract: Adolescent subjects with anxiety often present higher alcohol intake to diminish its symptoms compared to resilient subjects. Chronic alcohol intake is known to disrupt the sleep-wake cycle by decreasing both slow wave sleep (SWS) and rapid eye movement sleep (REM) on the resting phase of the circadian cycle, and increasing them during the active period. We have selectively bred two sublines from Sprague-Dawley rats that differ in their spontaneous yawning frequency. The high-yawning (HY) rats have a mean of 20 yawns/h, whereas low-yawning (LY) rats have just 2 yawns/h. The HY rats have shown resilient behavior and LY rats have shown an anxious trait when evaluated in different psychobiological anxiogenic tests. The aim of this study was to analyze free-choice alcohol intake in early and late adolescent HY and LY male rats and its sleep disturbance on adulthood. We used six male rats of each subline at 35-40 and 55-60 postnatal days (PND) of age for early and late adolescence groups, respectively. Subjects were exposed to a 10% v/v ethanol solution and purified water *ad libitum* on a two-bottle protocol for 30 days. Alcohol and water intake were measured daily, and a fresh ethanol solution was prepared daily. At 90±5 PND, subjects were implanted for electroencephalographic (EEG), electromyographic (EMG) and electrooculographic (EOG) recordings to characterize the sleep-wake stages. There was no significant difference in alcohol intake and preference index on any of the groups. However, alcohol produced an increase in SWS ($P<0.05$) and REM ($P<0.05$) on HY in both early and late adolescence groups and a decrease of wake during the dark phase ($P<0.05$). Similarly, LY rats of early and late adolescence groups presented an increase of both SWS ($P<0.05$) and REM ($P<0.05$) on the dark phase. Additionally, the increase of SWS and REM were higher on LY rats of both age groups when compared to the HY rats ($P<0.05$) In conclusion, chronic alcohol intake at an early age has a disruptive effect on the sleep-wake cycle of HY and LY rats, which lasted until adult age even after alcohol exposure ended. The LY rat is an adequate model for anxiety and depression. Additionally, the HY rat is a model for resiliency. Therefore, LY rats are more susceptible to sleep-wake alterations compared to HY rats.

Disclosures: A. Fierro-Rojas: None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.07/FF1

Topic: G.07. Biological Rhythms and Sleep

Support: R01 NS078410

Title: Effect of Mbnl2 knockout on ability to recover from sleep loss

Authors: *C. G. M. CAPUNO[1], N. HOA[2], J. D. ARBOLEDA[3], B. S. PINTO[3], K. TAMAI[1], E. T. WANG[3], K. N. PAUL[1];
[1]UCLA, Los Angeles, CA; [2]California State University, Long Beach, Westminster, CA; [3]Dept. of Mol. Genet. & Microbiology, Ctr. for NeuroGenetics, Univ. of Florida, Gainesville, FL

Abstract: The Muscleblind-like proteins (*Mbnls*) regulate developmental alternative splicing, polyadenylation, and localization pathways. Impairments of *Mbnl* activity are causatively implicated in the etiology of myotonic dystrophies (DM). While *Mbnl1* primarily governs the shift from juvenile to mature muscle tissue isoforms, its paralog, *Mbnl2*, performs similar functions within the CNS in neurons. Dysregulation of *Mbnl2* introduces deficits to both memory and cognition, but also disrupts sleep--a severe detriment to both health and quality of life. Despite neurological dysfunction and sleep dysregulation being reported as the most debilitating characteristics of DM1 by over half of surveyed patients, the mechanisms underlying these features remain elusive. By employing a murine FVB-background *Mbnl2* KO model of DM, we hereby characterize these disruptions to sleep architecture, and evaluate susceptibility to and subsequent recovery from 6-hour sleep deprivation periods, either ZT 0-6 or 6-12, via EEG/EMG electrophysiology (n=15). Preliminary results demonstrate baseline elevated REM episode proportions (p=0.02) alongside nominal wakefulness and NREM architecture. Statistical analyses were conducted using Welch's t-test. *Mbnl2* KOs demonstrate moderate wake fragmentation, suggesting perturbations to standard circadian rhythms, as well as higher transition frequency between NREM and REM, or blurring between the two stages of sleep. We also observe narcolepsy-like symptoms of what appear to be direct transitions from wakefulness to REM sleep. These phenotypes are exacerbated under sleep loss conditions, indicating a phenomenon of homeostatic sleep dysregulation--a trend we seek to investigate further. Our findings yield insight both into the pathophysiology of DM and the mechanisms of sleep loss recovery and sleep homeostasis as a whole. We are interested in further examining shifts in EEG spectral power attributable to this mutation under both baseline and sleep deprivation conditions, which will serve to elucidate better the contributions of *Mbnl2* to sleep and circadian regulation as they relate to holistic human health and disease resolution.

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Poster

PSTR404: Interaction Between Stressors and Sleep

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Support: NIH Grant R00NR014369
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Title: Sleep fragmentation increases cortical activation and blood pressure during slow wave sleep in rats

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Abstract: Introduction: Insufficient or disrupted sleep may increase the risk for hypertensive diseases. Electroencephalogram (EEG) spectral analysis quantifies signal power across frequency bands, providing a measure of sleep architecture. Low-frequency oscillations correlate with the depth of sleep; high-frequency oscillations contribute to vigilance. We hypothesize that sleep fragmentation (SF) decreases sleep depth and increases vigilance during slow wave sleep (SWS) resulting in increased blood pressure (BP) during SWS.
Methods: Male Wistar-Kyoto rats were implanted with transmitters to record BP and cortical EEG (Data Sciences International [DSI]). Light/dark cycle was maintained with Zeitgeber Time (ZT) 0-12, and ZT 12-24. Rats were randomized to 8 hours (ZT 1-9) of daily SF via a mechanical sweeper bar (n = 8) or undisturbed controls (n = 7). After acclimation to the SF chambers (Lafayette Neuroscience), EEG and BP were recorded over 3 days of baseline and 28 days of intervention. Mean arterial pressure (MAP) and relative EEG delta (0-4 Hz) and beta (16-24 Hz) power during SWS were calculated using 10-second epochs (NeuroScore, DSI).To analyze variables across multiple timepoints, mixed-effects models were used.
Results: SF affected SWS duration (group × time p <.0001) and sleep quality over time, as reflected by changes in EEG delta (p =.024) and beta power (p <.001) during SWS. Although SWS duration did not differ between SF and control groups after day 7, EEG spectral power analyses indicated worsening sleep quality with continued SF. By day 28, SF animals showed lower delta power (27.0 ± 6.5 vs. 42.5 ± 10.0; p =.022) and higher beta power (15.6 ± 2.7 vs. 10.2 ± 2.3; p = .018) compared to controls. MAP during SWS was also significantly influenced by SF over time (group × time p < .0001), with SF rats exhibiting higher MAP than controls on day 28 (106.45 ± 1.21 vs. 99.21 ± 4.74; p = .016). Across groups, differential associations between SWS duration with MAP, and EEG beta power with MAP occurred by treatment group over time (group × time p =.011 and p =.004).
Conclusion: Our findings demonstrate that SF alters sleep architecture and BP. SWS duration was similar between groups at later stages of SF, but differences in sleep quality, reflected by increased cortical arousal during SWS, contributed to differential BP responses during sleep between SF and control rats. This model has translational relevance for understanding cardiovascular risk associated with poor-quality sleep, such as in sleep disorders, chronic illness, or shift work. Ongoing work is evaluating whether changes in sleep quality during SF are linked to sustained BP elevations during bar-off periods.

Disclosures: L. Hentig: None. **K.A. Maki:** None. **J. Barb:** None. **L. Yang:** None. **A.M. Fink:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.09/FF3

Topic: G.07. Biological Rhythms and Sleep

Support: CONAHCYT CB2023-24-337
UAM-Iztapalapa 14407025

Title: Participation of M_1muscarinic Acetylcholine receptors in the regulation of memory in REM sleep deprived rats

Authors: R. MARTINEZ-GONZALEZ, R. ORTEGA-GOMEZ, ***A. JIMENEZ-ANGUIANO**; UAM-Iztapalapa, Mexico City, Mexico

Abstract: Acetylcholine (ACh) and muscarinic ACh receptors (mAChR) are involved in the regulation of the sleep wake cycle and the memory. However, it is still unknown the selective participation of mAChR during the Rapid Eye Movement (REM) sleep deprivation (REMSD) and in the recovery period on episodic memory. Therefore, the objective of this study was to evaluate the effect of M_1 mAChR in the regulation of declarative memory (DM) with the Novel Object Recognition test (NOR test). Male Wistar rats (250-270 g) were anesthetized and implanted with a cannula in the dorsal hippocampus (P=3.8, L=2.2, V=3.2). After one week the animals were randomly placed in the following groups (n=10): 1. Control, 2. Antagonist M_1 mAChR-Pirenzepine (PIRENZ-0.35 ug, in 0.2 ul of saline), 3. Agonist M_1 mAChR-BQCA (BQCA-0.35 ug/0.2 ul of saline), 4. 24 h REMSD, 5. 24 h REMSD + PIRENZ-0.35 ug/0.2 ul sal), 6. 24 h REMSD + BQCA-0.35 ug/0.2 ul sal), 7. 72 h REMSD, 8. 72 h REMSD + PIRENZ-0.35 ug/0.2 ul sal, 9. 72 REMSD + BQCA-0.35 ug/0.2 ul sal). Animals were REMSD using the modified multi-platform method. At the end of REMSD and, 7 and 14 days during the recovery period (RP) the animals were evaluated using the NOR test. The results obtained showed that 72 h REMSD and PIRENZ caused the greatest alterations affecting performance in the NOR test. BQCA improves the performance of the NOR test in control group and in the rats with REMSD. RP at 14 days had a beneficial effect in the NOR test. From these results we suggest that differential REMSD and M_1 mAChR have an impact in the correct learning and produces degradation in DM.

Disclosures: R. Martinez-Gonzalez: None. **R. Ortega-Gomez:** None. **A. Jimenez-Anguiano:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.10/FF4

Topic: G.07. Biological Rhythms and Sleep

Title: Sleep deprivation changes social behavior in African cichlid fish (Astatotilapia burtoni)

Authors: *B. M. PEREZ[1], D. SHOR[2], T. PREUSS[4], A. MARTASHVILI[3], M. EL MASRY[6], A. ADELINE[5];
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Abstract: Sleep deprivation disrupts cognitive and emotional processes, yet its effects on social behavior remain poorly understood despite the critical role social interactions play in group stability and individual fitness. To address this gap, we examined how sleep loss alters social behaviors in *Astatotilapia burtoni*, an African cichlid species characterized by complex social hierarchies and quantifiable aggressive interactions such as chases. Stable mixed-sex groups (N = 22) were used to assess behavioral changes under controlled lighting conditions. Initially, fish were maintained on a standard 12:12 light/dark cycle to establish baseline social dynamics. Partial sleep deprivation was induced by extending the light phase to 18 hours with a shortened 6-hour dark phase (18:6 light/dark), a manipulation that did not significantly alter chase frequency relative to controls (cont. 211 ±32 SEM; PSD = 172 ±11 SEM; $p = 0.44$; DF=2; t-match pairs). In contrast, total sleep deprivation involved 18 hours of constant light followed by 6 hours of pulsating light (4 min on, 1 min off), producing a marked increase in aggressive chases (cont. 224 ±14 SEM; TSD = 258 ±14 SEM; $p = 0.03$; DF=2; t-match pairs). To explore the role of social status in modulating sleep deprivation effects, 12 males were individually marked with dye and housed with 12 females, allowing longitudinal behavioral scoring. Dominance indices (DI) were calculated based on ethogram-derived observations. Preliminary analyses revealed that sleep deprivation disproportionately increased chase behavior in low DI in males. These findings imply that sleep loss may destabilize established social hierarchies, elevating aggression and potentially impacting group cohesion and fitness. Overall, this study advances understanding of how sleep deprivation influences complex social behaviors in group-living vertebrates, highlighting the need for further research on ecological and evolutionary consequences.

Disclosures: B.M. Perez: None. **D. Shor:** None. **T. Preuss:** None. **A. Martashvili:** None. **M. El Masry:** None. **A. Adeline:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.11/FF5

Topic: G.07. Biological Rhythms and Sleep

Support: 1U01NS126562-01

Title: Zebrafish sleep displays distinct sub-states

Authors: *R. TRIPATHI[1], G. OIKONOMOU[3], D. PROBER[4], G. J. GOODHILL[2];
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Abstract: Sleep is an essential behavior with distinct sub-states that serve different functions. While mammals exhibit well-defined sleep sub-states, it remains unclear to what extent such differentiation exists across the animal kingdom. Here we show, using long-term behavioral imaging combined with Hidden Markov Modeling, that larval zebrafish display distinct deep and light sleep sub-states. Although both sub-states occur primarily at night, they differ in their responsiveness to sleep deprivation and arousing stimuli. Moreover, the proportions of deep and light sleep are selectively altered by genetic and pharmacological manipulations of melatonin, serotonin, and norepinephrine pathways, offering new insights into how these neuromodulators shape sleep architecture. These results support zebrafish as a tractable model for dissecting the regulation and function of sleep sub-states. More broadly, they demonstrate that structured, multi-state sleep is a conserved feature of vertebrate behavior.

Disclosures: R. Tripathi: None. **G. Oikonomou:** None. **D. Prober:** None. **G.J. Goodhill:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.12/FF6

Topic: G.07. Biological Rhythms and Sleep

Title: The brain changes during sleep dysfunction caused by breast cancer

Authors: *C. HUNG[1], A. M. GOMEZ[2], J. C. BORNIGER[3];
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Abstract: Sleep problems are one of the most common symptoms in patients with cancer. Cancer impairs sleep quality, and poor sleep is also associated with cancer progression. The survival rate of cancer patients with bad sleep quality is lower than patients without sleep problems. These sleep problems persist even when controlling for hormone levels, surgery, or chemotherapy exposure. This suggests that the cancer itself may cause the sleep problems. Furthermore, even among cancer patients that survive for over 10 years, half still suffer from enduring sleep problems. These all indicate that cancer causes long term brain function change, but the mechanism of this is not well-identified. To identify how cancer alters brain function and sleep, we injected syngeneic EO771 breast cancer cells orthotopically into the 4th mammary gland and then compared the brain activity and sleep changes among the healthy (control), tumor-experienced (had cancer but removed), and tumor-bearing mice. Brain-wide neuronal

activity was assessed by quantifying cFos immunoreactive neurons, a neuronal activity marker in the brain. The cFos results revealed a that there were many differentially active nuclei (showing more cFos) across the brain in each group. Among all brain regions, 'hotspots' were identified in the hypothalamus, particularly the posterior division and periventricular area. These areas are mainly comprised of excitatory ($Vglut^{2+}$) neurons and project to several sleep-wake regulating areas, such as the lateral hypothalamus and periaqueductal gray. We will provide new data on identified regions and their relationship with sleep problems in cancer.

Disclosures: C. Hung: None. **A.M. Gomez:** None. **J.C. Borniger:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.13/FF7

Topic: G.07. Biological Rhythms and Sleep

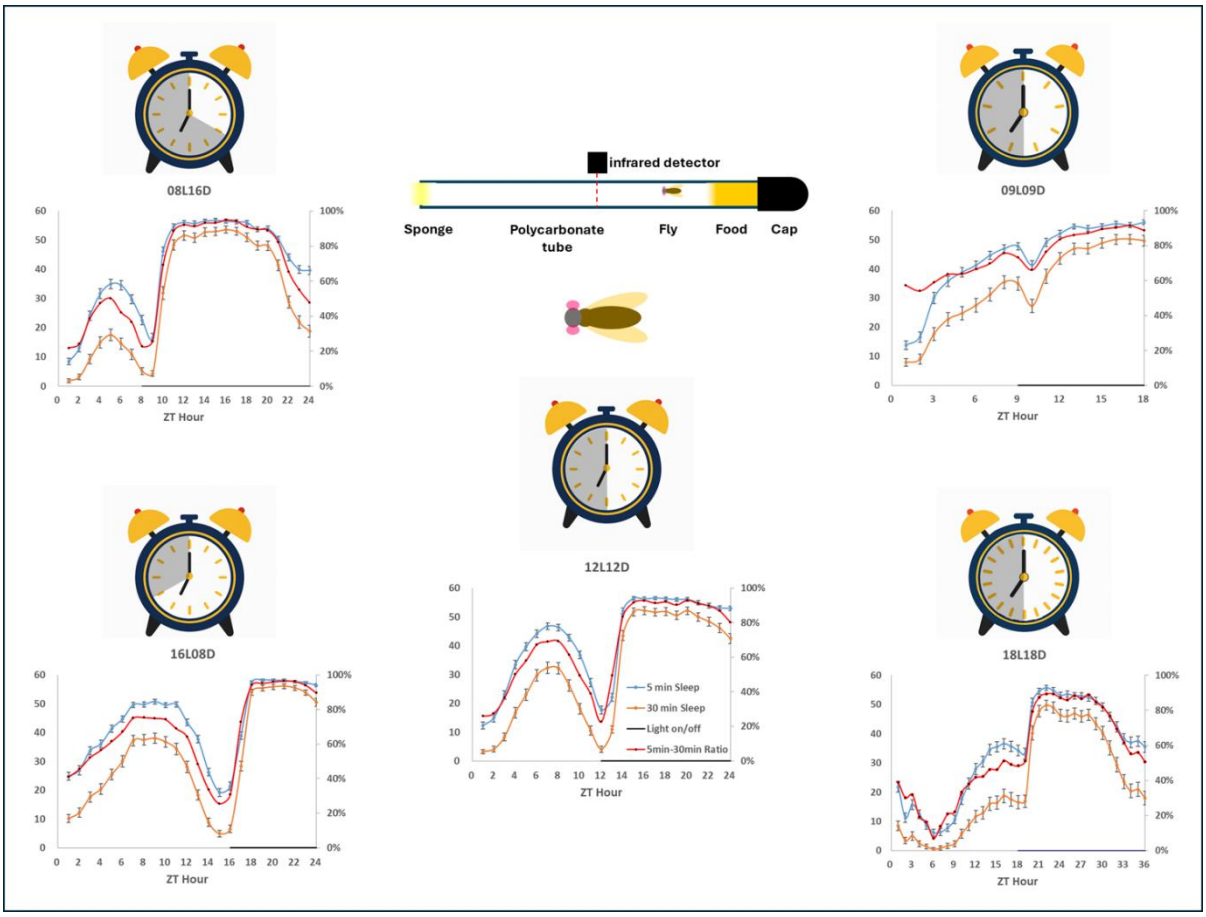
Support: UW-Madison L&S Scholarship
UW-Madison Hilldale Undergraduate/Faculty Research Fellowship
UW-Madison School of Pharmacy General Scholarship

Title: The Effect of Alternative Environmental Light-Dark Cycles on Sleep Profile

Authors: *E. CUI;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Sleep is a universal physiological activity across species, and its pattern is largely shaped by the environmental light-dark cycle. Most sleep research in various animal models focused on the standard 12:12 light-dark cycle or constant light/dark conditions, leaving the mechanism underlying adaptation to seasonal variations in light-dark cycles underexplored. Here, we measured sleep and CREB activity profiles in Drosophila melanogaster under multiple 24-hour-based non-12:12 light-dark cycles to investigate the mechanism for adapting to non-standard light-dark cycles. The sleep profiles under these cycles tend to converge toward those observed in constant light/darkness conditions, depending on the light-dark ratio. Notably, both the timing and duration of the longest sleep bout in a day also depend on the light-dark cycle. However, several key sleep features under the 12:12 light-dark cycle remain intact. With these experimental data, we developed a theoretical model to simulate sleep architecture under other 24-hour-based cycles. Interestingly, the CREB activity measured in the mushroom body, using the UAS-GAL4 and FLP-FRT systems, reflects the light-dark cycle and other observed sleep profiles. Furthermore, nighttime sleep under light-dark cycles with a longer nighttime period exhibited reduced functional importance, as indicated by decreased sleep rebound following sleep deprivation. Lastly, the observation that many sleep features under 24-hour-based light-dark cycles do not generalize under non-24-hour-based light-dark cycles (e.g., 9-hour-light-9-

hour-dark) supports the “escape from light” hypothesis, suggesting a co-evolution of circadian systems with naturally occurring environmental light-dark cycles.



Disclosures: E. Cui: None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.14/FF8

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R01 AG083268

Title: Dysfunctional Sleep in Young Ts66Yah Down Syndrome Model Mice

Authors: *R. A. MILSTEAD[1,2], B. FREIGASSNER[1], S. ZOU[1], C. BORSKI[1], P. CAIN[1], C. HOEFFER, Jr.[1,2];
[1]Univ. of Colorado, Boulder, Boulder, CO; [2]Linda Crnic Inst. for Down Syndrome, Aurora, CO

Abstract: Approximately 60% of Down syndrome (DS) individuals have sleep disturbances. Because sleep is a critical process for memory consolidation and other cognitive functions, sleep disturbances may contribute to cognitive deficits in DS. Ts65Dn mice, a DS model, exhibit increased wakefulness and reduced non-rapid eye movements sleep (NREMS), analogous to sleep disturbances in DS patients. We examined sleep in another DS model, Dp(16)1Yey (Dp16) mice. We described the sleep architecture and EEG characteristics in aged Dp16 mice and reported that disturbed sleep is characterized by increased wakefulness and decreased NREMS, consistent with DS individuals. Disrupted sleep is also prevalent in individuals with Alzheimer's disease (AD), with some estimates as high as 66% of patients exhibiting some form of sleep disturbance. Nighttime sleep disturbances include reduced NREMS, reduced rapid eye movement sleep (REMS), and decreased sleep efficiency. Additionally, nighttime sleep is fragmented, characterized by increased awakenings and greater overall wakefulness. Sleep disturbances often occur early in the disease, before clinical symptoms of cognitive impairment manifest, and worsen with disease progression. While the Ts65Dn model has been used for years, it contains triplicated genes that are not found on human chromosome 21 (Hsa21), raising questions about its validity as a DS model. Recently, a new DS mouse model, known as the Ts66Yah, was introduced. This new Ts66Yah strain is very similar to the Ts65Dn animals, but it lacks non-Hsa21 genes in the mini-chromosome. Using this new model, we measured changes in sleep phases and EEG frequency power using surgically implanted head-mounted EEG recordings. We found that young Ts66Yah animals exhibit a sleep dysfunction phenotype similar to that of aged Dp16 animals, characterized by increased wakefulness at the expense of NREM sleep. This phenotype appears much earlier in Ts66Yah animals compared to Dp16 animals (~3 months for Ts66Yah vs. ~10 months for Dp16), making the Ts66Yah a more clinically and translationally relevant model for studying sleep dysfunction in DS. Taken together, this study suggests that Ts66Yah DS model mice will be a valuable tool for disentangling the complex role of sleep dysfunction in DS and its progression to AD.

Disclosures: R.A. Milstead: None. **B. Freigassner:** None. **S. zou:** None. **C. Borski:** None. **P. Cain:** None. **C. Hoeffer:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.15/FF9

Topic: G.07. Biological Rhythms and Sleep

Title: The Effect of Social Jetlag on Caffeine Intake and Pain Sensitivity

Authors: *M. KELLEY[1], E. SKAER[1], M. IMAN[1], A. SABBAGH[1], Y. SHEN[2], A.-M. CHANG[2], W. J. HORTON[2];
[2]Biobehavioral Hlth., [1]Pennsylvania State Univ., University Park, PA

Abstract: One-third of college students report obtaining fewer than seven hours of sleep per night, the minimum recommended amount for adults. Sleep and pain share a well-documented bidirectional relationship, and college students have a surprisingly high rate of acute pain. However, the mechanisms that underpin this cycle remain poorly understood. One possible factor is social jetlag, defined as the mismatch between a person's natural sleep-wake cycle and their social schedule. This misalignment may interfere with both sleep regulation and pain perception, potentially perpetuating a cycle of poor sleep, increasing reported pain. In addition, caffeine consumption has been shown to impact sleep quality and quantity. Previous work has identified a link with caffeine intake and pain sensation, which we have recapitulated in our sample ($r = 0.167$, $p < 0.001$); therefore, we included social jetlag as a possible moderating factor in the caffeine-pain relationship. We hypothesized that greater social jetlag would be associated with increased pain outcomes among college-aged students, and that caffeine consumption may contribute to or exacerbate this relationship.
To examine these hypotheses, data were collected from students aged 18 to 24 ($n = 32$), who completed daily surveys over one week during their normal routines. Participants completed the Munich Chronotype Questionnaire (MCTQ) to assess social jetlag, as well as daily self-reports of pain and caffeine intake. Social jetlag was calculated as the difference in mid-sleep time between free days and school or workdays. Preliminary analyses ($n = 19$) using a simple correlation between social jetlag and self-reported pain failed to show significant results ($r = 0.089$, $p = 0.214$). Although these findings do not currently support an association, they may reflect a small effect size and/or limited power due to the reduced sample size. As data collection is ongoing, we plan to conduct additional analyses with a larger sample to further assess whether social jetlag may serve as a contributing factor in the bidirectional sleep-pain relationship. In addition, we are conducting mediation analyses and exploring the potential role of caffeine intake.
Understanding the impact of social jetlag on the sleep-pain relationship may offer valuable insights for public health strategies aimed at improving sleep and reducing pain in young adults. With circadian disruption common among college students, these findings can inform targeted interventions centered on education, behavior change, and preventive care. This research reinforces the importance of addressing modifiable risk factors to advance health promotion and reduce disparities.

Disclosures: M. Kelley: None. **E. Skaer:** None. **M. Iman:** None. **A. Sabbagh:** None. **W.J. Horton:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.16/FF10

Topic: G.07. Biological Rhythms and Sleep

Title: Curcumin Attenuates Circadian Disruption-Induced Neuroimmune Activation via Modulation of Cytokines and the Gut-Brain Axis

Authors: T. MANDYAM[1], ***S. SIMPSON**[2];
[1]Anethesia, Univ. of California San Diego, La Jolla, CA; [2]Anesthesia, UCSD, La Jolla, CA

Abstract: Circadian disruption is a potent driver of neuroinflammatory signaling and has been implicated in a range of neuropsychiatric and neurodegenerative conditions. However, therapeutic strategies that safely mitigate inflammation induced by circadian rhythm disturbance remain limited. In this study, we investigated the anti-inflammatory potential of curcumin, a plant-derived polyphenol with known neuroprotective properties, in a rat model of systemic circadian misalignment.Rats were subjected to a chronic 12-hour light/dark phase reversal (12L:12D photoperiod shift) to induce circadian desynchrony and were treated daily with either oral curcumin (40 mg/kg in almond butter vehicle) or vehicle alone. Inflammatory responses were quantified by plasma pro-inflammatory cytokines (TNF-α, IL-6, CXCL1; ELISA), gut-associated lymphoid tissue size, and gut microbial composition via metagenomic sequencing.Curcumin treatment significantly reduced systemic markers of inflammation: plasma cytokines were reduced by ~70%, Peyer's patch size by ~50%, and significant reduction in the relative abundance of pro-inflammatory microbiota compared to vehicle controls. These findings suggest that curcumin modulates neuroimmune signaling through peripheral cytokine suppression and restoration of gut microbial homeostasis—key components of the gut-brain axis. This work supports curcumin's potential as a non-pharmacologic strategy to ameliorate neuroinflammation triggered by circadian rhythm disruption, with implications for shift work-associated disorders, mood dysregulation, and neurodegenerative disease risk.

Disclosures: T. Mandyam: None. **S. Simpson:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.17/FF11

Topic: G.07. Biological Rhythms and Sleep

Support: NIH grant R01AT012707

Title: Effects of unpredictable chronic mild stress on sleep and behavior in the mouse

Authors: *F. SQUARCIO, J. MCNERNEY, G. TONONI, C. CIRELLI;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Rodent studies found that chronic stress has complex effects on both the macrostructure and the microstructure of sleep, with variable results depending on the specific

paradigm. Unpredictable chronic mild stress (UCMS) was found to increase REM sleep and disrupt NREM sleep continuity, while chronic isolation stress and social defeat stress impair NREM sleep duration and slow wave activity (SWA). Both UCMS and social defeat stress can cause "depressive" symptoms (decreased sucrose preference, increased social avoidance) and anxiety behavior (reduced exploration in the open field), but only in a subset of animals. The extent to which stress-induced sleep changes can predict the development of these symptoms remains unclear. SWA in NREM sleep is linked to several of the restorative properties of sleep, from synaptic downselection to memory consolidation. The extent to which stress-induced sleep changes can compromise sleep's ability to support these restorative functions is also unclear. In this ongoing study, we expose C57BL/6J mice (so far, 5 males, 5 females, 20.3 ± 0.2 weeks old) to a 4-week UCMS protocol. Different behavioral tests are used to evaluate anxiety and depressive symptoms before and after UCMS. 24-hour sleep recordings before and after UCMS are performed to assess changes in sleep architecture. After UCMS, mice are either allowed to sleep or sleep deprived for 4 hours before brain collection. Ultrastructural analyses using serial electron microscopy will test whether chronic stress prevents sleep's restorative effects on synaptic size and astrocytic function. Preliminary analyses show that after UCMS, all animals have increased anxiety and depressive symptoms and spend more time in both NREM and REM sleep, but with large interindividual variability. Mice also show an increase in slow wave energy (SWE, SWA x NREM sleep duration) and theta energy in REM sleep (theta x REM sleep duration). A trend towards a negative correlation ($r = -0.31$, $p = 0.053$) is present between SWE in the post-UCMS recording and the severity of depressive symptoms. Thus, after UCMS, higher SWE is associated with lower depression severity.

Disclosures: F. Squarcio: None. **J. McNerney:** None. **G. Tononi:** None. **C. Cirelli:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.18/FF12

Topic: G.07. Biological Rhythms and Sleep

Title: Insights into Sleep Architecture and EEG Spectral Profiles During Postoperative Recovery in Group-Housed Mice.

Authors: *T. VELIE[1], K. NICHOLS[2], J. VAN HEE[3], K. HOLLIDAY-WHITE[2], J. ALVAREZ[4];
[1]Data Sci. Intl., St. Paul, MN; [2]Data Sci. Intl., New Brighton, MN; [3]Data Sci. Intl., New Brigton, MN; [4]Data Sci. Intl., Holliston, MA

Abstract: Studying physiological parameters during post-surgical recovery is critical for gaining insights into animal health and determining readiness for experimentation. However, technological restrictions pose practical challenges when conducting experiments with socially housed animals. To address this, we developed a novel telemetry system, SoHo™, capable of

simultaneously collecting electroencephalogram (EEG), electromyogram (EMG), body temperature, and locomotor activity in group-housed small animals. This study evaluates the efficacy of the SoHo™ telemetry platform to wirelessly assess sleep and EEG spectral profiles in group-housed mice. C57BL/6 female mice (22 weeks old; N=5) were surgically instrumented subcutaneously with the SoHo™ telemetry transmitters (model X02) and group-housed under a standardized 12-hour dark/light cycle. Synchronized EEG, EMG, body temperature, gross locomotor activity, and video data were continuously collected during 12 days of post-operative recovery. Following surgery (day 0), the average percentage of slow wave sleep (SWS) was 32.9 ± 15.5% and paradoxical sleep (PS) was 11.2 ± 6.1%. By day 12, SWS decreased to 26.2 ± 9.7% and PS decreased to 5.2 ± 0.9%. EEG Delta power was 4693 ± 276 μV^2 (dark phase) and 3843 ± 742 μV^2 (light phase) on day 0 and decreased to 2581 ± 410 μV^2 (dark phase) and 3019 ± 359 μV^2 (light phase) on day 12. EEG Theta power was 1802 ± 405 μV^2 (dark phase) and 2535 ± 516 μV^2 (light phase) on day 0 and 1838 ± 434 μV^2 (dark phase) and 2446 ± 407 μV^2 (light phase) on day 12. EEG Alpha, Beta, and Gamma power bands did not show statistical differences. Subcutaneous temperature readings were 35.9 ± 0.4 ºC (dark phase) and 35.7 ± 0.2 ºC (light phase) on day 0, and 36.3 ± 0.3 ºC (dark phase) and 35.6 ± 0.3 ºC (light phase) on day 12. Post-surgery, average gross locomotor activity (calculated as counts per minute; cpm) was 13181 ± 509 cpm (dark phase) and 81707 ± 8161 cpm (light phase). By day 12, gross locomotor activity was 192463 ± 29604 cpm (dark phase) and 58248 ± 5751 cpm (light phase). The data demonstrates that SoHo™ telemetry is a robust tool for evaluating EEG, EMG, temperature, and activity variations in socially housed mice. Future studies using pain interventions and housing strategies will help further characterize EEG dynamics during recovery.

Disclosures: T. Velie: A. Employment/Salary (full or part-time): Employee. **K. Nichols:** A. Employment/Salary (full or part-time): Employee. **J. Van Hee:** A. Employment/Salary (full or part-time): Employee. **K. Holliday-White:** A. Employment/Salary (full or part-time): Employee. **J. Alvarez:** A. Employment/Salary (full or part-time): Employee.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.19/GG1

Topic: G.07. Biological Rhythms and Sleep

Support: MH 60670

Title: Dynamic sleep patterns following traumatic stress exposure, compared with following post-fear learning and exposure to non-traumatic novelty learning reveal nervous system adaptability to psychological stress

Authors: *Y. CABRERA[1], G. R. POE[2];
[1]UCLA, Los Angeles, CA; [2]Dept. of Integrative Biol. and Physiol., UCLA Chapter, Los Angeles, CA

Abstract: Dynamic sleep patterns following traumatic stress exposure, compared with following post-fear learning and exposure to non-traumatic novelty learning reveal nervous system adaptability to psychological stress Abstract

Disclosures: Y. Cabrera: None. **G.R. Poe:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.20/GG2

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant R01NS132672
DoD Grant W81XWH-14-1-0467
DoD Grant W81XWH-14-1-384

Title: Sleep-based biomarkers enable early detection of traumatic brain injury in mice

Authors: *G. S. MANNINO[1], *G. S. MANNINO[2], R. K. ROWE[3];
[1]Univ. of Colorado Boulder, Boulder CO, USA | Stanford University, Stanford CA, USA, Boulder, CO; [2]Univ. of Colorado Boulder, Boulder CO, USA, Boulder, CO; [3]Univ. of Colorado Boulder, Boulder, CO

Abstract: Sleep fragmentation - defined by frequent sleep-wake transitions - is a hallmark of various neurological conditions, including traumatic brain injury (TBI). While sleep duration and architecture have been extensively studied, quantifying sleep-wake transitions offers a novel approach to classifying injury severity and assessing sleep stability following various experimental TBI models. Here, we introduce a noninvasive method for detecting sleep-wake transitions using a piezoelectric cage system and assess its utility in characterizing baseline sleep architecture and post-injury sleep disturbances. We developed an automated pipeline using a custom Excel VBA macro to efficiently process transition data, enabling high-throughput, noninvasive analysis of sleep fragmentation. This approach was applied to assess baseline sex differences in sleep stability and to investigate sleep-wake transitions as a biomarker for neurological outcomes after TBI. Under normal physiological conditions, female mice exhibited significantly more sleep-wake transitions than males (n=32; p=0.0175), emphasizing inherent sex differences in sleep stability. Next, we investigated the prognostic value of sleep-wake transitions by analyzing post-injury sleep following mild and moderate midline fluid percussion injury (mFPI) in adult mixed sex mice (n=94). Moderately injured mice exhibited significantly greater sleep fragmentation than those with mild TBI (p<0.0001), suggesting that transition frequency scales with injury severity and could serve as a biomarker for stratifying patient outcomes. Finally, in adult mixed-sex mice, we assessed the diagnostic potential of sleep-wake transitions across three distinct TBI models—controlled cortical impact (CCI), closed head injury (CHI), and mFPI—which replicate the heterogeneity of clinical TBI (n=102). Principal

Component Analysis (PCA) of 72-hour post-injury sleep data revealed distinct clustering of TBI and sham groups (PC1: $p<0.001$), demonstrating that sleep-wake transitions differentiate injured from uninjured subjects. The ability to quantify sleep-wake transitions without requiring surgical implantation of recording electrodes expands the applicability of this method to diverse experimental paradigms, including studies of circadian rhythms, neurodegenerative diseases, and pharmacological interventions. Moreover, our findings establish sleep-wake transitions as a scalable, non-invasive biomarker for TBI classification and severity assessment, offering potential applications for early diagnosis and individualized treatment strategies in neurotrauma care.

Disclosures: G.S. Mannino: None. **G.S. Mannino:** Other; help. **R.K. Rowe:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.21/GG3

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant AA028175-01
VA Merit Award I01BX002661
VA Merit Award I01BX006240-0

Title: Blast-induced mTBI alters spontaneous sleep wakefulness and impairs sleep homeostasis

Authors: *R. SHARMA[1,2], **M. M. THAKKAR**[4], M. PARIKH[2], I. PATHAK[3], B. OSBURN[5], L. MA[6], T. ROLD[7], J. SMITH[6];
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Abstract: Mild Traumatic Brain Injury (mTBI) is most common in combat veterans, often resulted by blast exposure. One of the persistent symptoms of mTBI is disrupted sleep. The objective of this study is to investigate the effects of open field blast exposure on sleep wakefulness architecture. We hypothesized that the open field blast mouse model of mTBI alters spontaneous sleep-wakefulness and impairs sleep homeostasis. *Blast Protocol: -* Adult male (n# 12) mice were subjected to open-field blast exposure to generate a reproducible primary blast wave. Animals were assigned to three groups: (1) single blast (1x) exposure, (2) double blast (2x) exposure, and (3) No Injury Control (NIC). Sham animals underwent identical handling protocols without detonation. *Surgery: -* Animals(n=4/group) were transferred to the surgery room. Three cortical EEG electrodes were positioned on the skull, and three EMG electrodes were placed into the nuchal muscles for the sleep recording (i.e., ZT0= 7:00 AM). Animals were kept in constant light: dark conditions. Spontaneous sleep-wakefulness was classified before the sleep deprivation experiment. *Sleep deprivation Protocol: - To* evaluate the effects on sleep

homeostasis, mice were subjected to gentle handling for the duration of 6 hours, spanning the second half of the light period (ZT6-12; 1 PM to 7 PM). This protocol was implemented to prevent sleep onset without inducing excessive stress, ensuring that the animals remain awake while maintaining minimal disruption. Spontaneous sleep-wakefulness was significantly disrupted in mTBI mice compared to the control. During sleep deprivation, mTBI mice failed to show an increase in wake theta power, indicate failure in homeostasis build-up during sleep deprivation. mTBI mice showed a reduced delta power rebound after sleep deprivation. During Sleep recovery, Increased wake and disrupted NREM were observed. Blast-induced mTBI disrupts spontaneous sleep-wakefulness and impairs sleep recovery following sleep deprivation.

Disclosures: R. Sharma: None. **M.M. Thakkar:** None. **M. Parikh:** None. **I. Pathak:** None. **B. Osburn:** None. **L. Ma:** None. **T. Rold:** None. **J. Smith:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.22/GG4

Topic: G.07. Biological Rhythms and Sleep

Support: Veterans Administration - OFBC data submission for merit
National Institute of Health (through the RO1 grant; AA028175-01),
Veterans Administration (through the Merit Research Award; I01BX002661 and I01BX006240-0)
MU Research Council (URC-22-006)

Title: Blast-induced mTBI causes chronic sleep disruption and spatial memory impairment

Authors: *M. PARIKH[1], I. PATHAK[2], R. SHARMA[2], B. OSBURN[2], T. ROLD[3], J. SMITH[4], L. MA[5], M. M. THAKKAR[6];
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Abstract: Background: Sleep disturbances are highly prevalent in mTBI (mild traumatic brain injury) patients. Previous studies have also shown the cognitive decline in TBI patients. However, the underlying mechanism is yet to be identified. We hypothesized that *mTBI induces chronic sleep disturbances and spatial memory impairment.* **Methods:** Adult male (n#27) mice were subjected to open-field blast exposure. Animals were assigned to three groups: (1) one blast (1x) exposure, (2) two blast (2x) exposure, and (3) No injury Control (NIC). Cohort#1 (N=4/group) male mice were transferred to the surgery room. Local field potential electrodes were implanted into the hippocampal CA1 region (coordinates: AP -2.0 mm, ML ±1.5 mm, DV -1.3 mm from bregma). 3 cortical EEG electrodes were implanted on the skull, and 3 EMG

electrodes were placed into the nuchal muscles. Spontaneous sleep wakefulness was recorded for 24 hours.Cohort#2 (N= 5/group) In vivo magnetic resonance imaging (MRI) was performed on a 7T system with a cryoprobe under 1.5% isoflurane anesthesia. T2-weighted MRI provided anatomical details. Cerebral blood flow (CBF) perfusion was quantified using arterial spin labeling (ASL) MRI. Diffusion tensor imaging (DTI) was performed with 30 directions and b = 1250 s/mm² to evaluate microstructural changes. Proton magnetic resonance spectroscopy (^{1}H-MRS) was acquired in the hippocampus and cortex for metabolite quantification. *Behavior Test: -* All animals were tested for spatial memory using the Novel Object Location Task (NORT) at the onset of the active period. All the parameters were measured at a 7-month point from the blast to assess the chronic effect of blast induced-mTBI. **Result:***Cohort#1* Chronic sleep disturbances and significantly reduced wake theta power in hippocampal LFPs were observed in the double blast exposure group of mice, compared to both single blast exposure and control groups. Behavioral analyses revealed deficits in spatial memory in mTBI group.*Cohort#2* 2x blast-exposed males showed significantly decreased DTI quantitative anisotropy (QA) in the cortex and hippocampus compared to sham, and reduced QA in the right hippocampus when compared with the 1x group. MRS showed increased taurine in the cortex and hippocampus in the 2x group and increased cortical t-choline in both 1x and 2x groups. Cortical and hippocampal CBF was modestly reduced in the 2x group but did not reach statistical significance. **Conclusion:** mTBI-mice exhibited significant spatial impairment following chronic sleep disturbances, which was accompanied by a marked accumulation of taurine and t-choline in the hippocampus and cortex in the 2x group compared to controls.

Disclosures: M. Parikh: None. **I. Pathak:** None. **R. Sharma:** None. **B. Osburn:** None. **T. Rold:** None. **J. Smith:** None. **L. Ma:** None. **M.M. Thakkar:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.23/GG5

Topic: G.07. Biological Rhythms and Sleep

Support: DFG In 279/2-1
DFG Bo 854/18-1
ERC AdG 883098 Sleep-Balance

Title: Post-encoding sleep enables schema memory consolidation in the infant rat brain

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Abstract: Schemas are cognitive frameworks that allow rapid integration of new information into existing knowledge structures. While schema-related memory processes are well

characterized in adults, it remains unknown how in early development the brain can extract and consolidate structured knowledge. Here, we investigate the formation of schema memory in infant rats and evaluate whether post-encoding sleep supports its consolidation.
To assess schema memory, we adapted the object-place recognition paradigm to test rats at postnatal day (PD) 25. Animals were divided into three groups (n = 8 per group): Schema, No-Schema, and Sleep Deprivation. During encoding, the Schema group experienced eight learning episodes separated by 5-minute intervals, where one object remained consistently placed while the other alternated between two locations allowing abstraction of a spatial regularity. The No-Schema group encountered objects in random locations across trials, preventing rule formation. The Sleep Deprivation group underwent the same schema protocol but was kept awake for two hours following learning. Twenty-four hours later, memory was probed at PD26 by relocating the previously stable object to a novel position, breaking the spatial rule.
Only the Schema group showed significant preference for the displaced object, indicating successful rule abstraction and retrieval. In contrast, No-Schema and Sleep Deprivation groups showed no evidence of memory expression, suggesting that schema memory can emerge early in development but requires post-encoding sleep for consolidation.
We quantified cFos expression and assessed functional connectivity using graph-theoretical measures. The Schema group showed reduced degree centrality in hippocampal subregions CA1 and CA3 compared to Sleep Deprivation and No-Schema groups along with lower hippocampal betweenness centrality relative to null models, suggesting reduced hippocampal reliance. In contrast, the Sleep Deprivation group showed hyperconnected networks, possibly reflecting unspecific or inefficient consolidation processes.
To causally test hippocampal involvement, we will next use Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to inhibit hippocampal activity during post-learning sleep. Comparing these findings with adult data will help determine whether developing and mature brains use distinct systems to consolidate schema memory.

Disclosures: J. Fechner: None. **J. Born:** None. **M. Inostroza:** None.

Poster

PSTR404: Interaction Between Stressors and Sleep

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR404.24/GG6

Topic: G.07. Biological Rhythms and Sleep

Title: Sleep regulation by vagus nerve in response to inflammation

Authors: *K. IWASAKI[1], L. DE LECEA[2];
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Abstract: Sleep and inflammation are known to influence each other. Sleep deprivation elevates levels of pro-inflammatory cytokines in the blood, while pro-inflammatory cytokines promote

sleep. However, the pathways that mediate communication between peripheral inflammation and sleep-wake states remain largely unknown. Given that the sensory vagus nerve is activated by inflammation and provides negative feedback on circulating cytokine levels, I hypothesize that a specific subpopulation of vagal sensory neurons detects inflammatory signals and promotes sleep in response, thereby facilitating recovery from immune insults.To test whether activation of different subpopulations of neurons in the vagal complex is sufficient to induce sickness-related sleep, I employed the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) system in combination with electroencephalogram (EEG)-based sleep analysis. I found that activation of a specific subpopulation of vagal neurons that is activated by inflammation was sufficient to induce sleep, whereas other populations tested did not produce a sleep response upon activation. These findings suggest that an immune-responsive subpopulation of the vagus nerve plays a critical role in sleep production in an inflammatory state. This study will contribute to our understanding of how peripheral pro-inflammatory cytokines induce sleep and how inflammation-induced sleep promotes recovery from immune challenges.

Disclosures: K. Iwasaki: None. **L. de Lecea:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.01/GG7

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH Grant K99DA058709
NIH Grant R37DA033396
NIH Grant P30DA048736

Title: Rapid endogenous opioid dynamics in the dorsal striatum promote goal-directed action

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Abstract: Targeting the endogenous opioid dynorphin, via the kappa-opioid receptor (KOR) has shown promise in the treatment of multiple neuropsychiatric disorders including substance use disorder (SUD). A hallmark of SUDs is maladaptive goal-directed behavior, which is our ability to make causal associations between actions and rewarding outcomes. The dorsomedial striatum (DMS), where dynorphin is abundant, is critical for goal-directed behavior. Furthermore, enhanced dynorphin-KOR signaling and aberrant striatal activity are associated with the transition of recreational to persistent drug-seeking. Yet, how DMS dynorphin neuron activity, dynorphin release, and subsequent dynorphin-KOR signaling promote goal-directed behavior is unknown. Here, using *in vivo* two-photon calcium imaging of thousands of dynorphin neurons

when mice learn to perform actions to obtain rewarding outcomes, we observe an increase in dynorphin neuron recruitment that evolve discrete patterns of activity during behavior upon learning. Importantly, using an artificial recurrent neural network trained to predict engagement in goal-directed behavior, we show that dynorphin neuron activity specifically during the anticipation of the outcome promotes subsequent goal-directed action. Next, we assayed *in vivo* DMS dynorphin dynamics during goal-directed behavior using a novel genetically-encoded dynorphin biosensor. We observe that dynorphin tone increases upon learning, and is released specifically in anticipation of reward delivery upon learning. Furthermore, using a generalized linear model, we show that the magnitude of dynorphin release strongly correlates with subsequent goal-directed action engagement. In support of these findings, mimicking DMS dynorphin release during anticipation using optogenetics enhances goal-directed action without being inherently reinforcing, and is sensitive to KOR antagonism. Conversely, we find that conditional deletion of dynorphin production from the DMS decreases the learning and performance of goal-directed behavior, without affecting the innate preference for sucrose. Altogether, we reveal that dynorphin-KOR signaling in the DMS shapes goal-directed behavior, by dynamically promoting future goal-directed action at strikingly fast timescales.

Disclosures: R. Gowrishankar: None. **M. Hjort:** None. **L. Tian:** None. **M.R. Bruchas:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.02/GG8

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH grant 1P50MH136296-01

Title: The latent-cause framework for understanding addiction: role of the orexin/hypocretin system

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Abstract: When individuals are exposed to novel information, a critical question arises: is this information assimilated into an existing latent cause, or does it prompt the formation of a new one? For instance, when a previously rewarded behavior no longer yields reinforcement, individuals must infer whether this reflects a modification of an existing latent cause (one-cause model) or the emergence of a distinct latent cause (two-cause model). Our laboratory has previously demonstrated that the orexinergic/hypocretin system plays a pivotal role in regulating motivation towards a reward. Other studies have demonstrated that orexin neurons contribute to the encoding of reward-related memories, including through projections towards the hippocampus. This orexinergic pathway is involved in memory formation, extinction processes,

and reward-seeking behaviors, with signaling predominantly mediated by orexin receptor 1 (OxR1). Here, we investigated the role of OxR1 in encoding reward-associated memories within both a one-cause and two-cause models. Male rats were trained to lever press for sucrose in a specific environmental context (Box A), and then underwent extinction training (unrewarded lever pressing) in either Box A or in a distinct context (Box B). Animals were later tested for renewal in context A (hence, AAA vs ABA renewal paradigms). Latent cause inference predicts greater tendency to infer a single latent cause for all three stages of the experiment in AAA renewal, whereas ABA renewal is more likely to promote inference of two distinct latent causes. To examine the role of the orexin/hypocretin system in this process, we pharmacologically blocked OxR1 signaling using SB-334867 during extinction training and evaluated its impact on renewal both on day 1 and following 28 days of home-cage abstinence. Additionally, we used a chemogenetic approach to selectively manipulate orexinergic projections to the CA3 to further elucidate their role in context-dependent modulation of reward-seeking behavior. Our data show that orexin 1 receptor blockade during extinction increases renewal in the AAA group. This effect was not repeated after 28 days of abstinence. Injections of SB also facilitated extinction without impacting motivation, arousal or locomotor activity, suggesting an impact of orexin 1 receptor blockade on reward-associated memories. Altogether, our preliminary results suggest that orexin signaling regulates the inference of the latent cause. They also highlight orexin inputs in the hippocampus as a potential target to manipulate the recall of reward-associated memory.

Disclosures: D. De Sa Nogueira: None. **G.S. Aston-Jones:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.03/GG9

Topic: H.02. Reward and Appetitive Learning and Memory

Support: National Research Foundation of Korea.

Title: Investigation of Lateral Habenula Neural Circuits Underlying Compulsive Consumption

Authors: *S. CHOI[1], S. LEE[2], J. PYO[1], N. KOO[1], S. CHOI[1], T. KIM[1], J.-H. KIM[1];
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Abstract: Consumption is generally driven by homeostasis in response to nutritional needs. However, people also consume palatable foods based on preferences. Consumption of palatable foods could share many features with addictive behavior, particularly in the aspect of compulsion - the continuous intake despite negative consequences. Individuals affected by this compulsion often overeat foods, resulting in negative effects on their physical health, emotion, and overall well-being.
In our study, we aimed to investigate the neural circuits underlying the compulsive consumption. This concept was modeled in rodents by associating the palatable foods with negative stimuli

(strong bitter taste). Categorizing mice into resistant (compulsive) and sensitive (non-compulsive) groups, we identified distinct activation in lateral habenula (LHb) within the resistant group. Therefore, we proposed the potential role of the LHb in regulating compulsive consumption.
To pinpoint specific neuronal populations within the LHb involved in compulsive consumption, we conducted single-cell RNA sequencing and single-nucleus ATAC (Assay for Transposase-Accessible Chromatin) sequencing. Based on transcriptomic and epigenetic analysis, we identified a specific genetic marker within the activated neuronal population. We confirmed the functional role of the neural circuits through optogenetics and chemogenetics. We also observed the activity of these neural circuits during consumption. Our findings shed light on the neural mechanisms underlying compulsive consumption, offering valuable insight into various mental disorders characterized by abnormal consumption behaviors.

Disclosures: S. Choi: None. **S. Lee:** None. **J. Pyo:** None. **N. Koo:** None. **S. Choi:** None. **T. Kim:** None. **J. Kim:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.04/Web Only

Topic: H.04. Emotion

Support: NIH grant R21AG067024
NIH grant R01AG072893

Title: Behavioral and neural correlates of negative emotion processing in neurotypical drinkers: roles of alcohol use, anxiety, and sex differences

Authors: *S. CHAUDHARY[1], Y. CHEN[1], H. WONG[2], C.-S. R. LI[3];
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Abstract: Negative emotion is a key psychological factor contributing to alcohol use, and alcohol consumption often increases following negative emotion induction. Alcohol also alters brain responses during emotional processing. Notably, behavioral and neuroimaging studies have reported sex differences in responses to negative emotions, particularly in the context of anxiety, a factor implicated in the initiation, maintenance, and relapse of alcohol use disorder (AUD). To examine how alcohol use affects emotion processing across sexes and anxiety levels, we studied 150 adults (73 women) using the Alcohol Use Disorders Identification Test (AUDIT) and State-Trait Anxiety Inventory (STAI). Participants performed a Hariri task during fMRI, matching emotional images (12 negative, 12 neutral) while suppressing emotional reactivity. Faster reaction times (RTs) were interpreted as better emotion regulation. Brain data were collected using a 3T Siemens Trio and analyzed with SPM12. Men and women did not differ significantly

in age, drinking history, or task performance. However, a significant sex difference emerged in the association between AUDIT and RT (negative - neutral): higher AUDIT scores were linked to faster RTs in men but slower RTs in women. AUDIT also correlated with STAI scores in men, with a trend in women. Whole-brain full-factorial analysis revealed sex-specific AUDIT effects on activity in the middle temporal gyrus (MTG), supramarginal gyrus (SMG), and middle frontal gyrus (MFG), where activity increased with AUDIT in men but decreased in women. MTG and SMG activity also correlated with anxiety in men and partially mediated the AUDIT-anxiety relationship (22-24%). Connectivity analyses showed that, in men, MTG-MFG and SMG-MFG connectivity positively correlated with anxiety and moderated the relationship between anxiety and alcohol use. In contrast, in women, only MFG-cuneus connectivity correlated with anxiety, and no interaction effects emerged. In sum, men with greater alcohol use showed enhanced frontal regulatory activity and connectivity, possibly reflecting compensatory mechanisms linked to anxiety. In women, reduced frontal activation suggests impaired emotion regulation, but alcohol use and anxiety did not interact, indicating distinct neural pathways.

Disclosures: S. Chaudhary: None. **Y. Chen:** None. **H. Wong:** None. **C.R. Li:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.05/GG10

Topic: I.03. Decision Making

Support: Sloan Swartz Foundation
Burroughs Wellcome Fund
NIH F32MH132179
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Grave Endowment Scholarship
NIH R01NS113113

Title: Rate-based models of decision termination capture single-trial superior colliculus bursting dynamics

Authors: *L. BROWN[1], A. E. COMRIE[2], G. M. STINE[3], M. S. GOLDMAN[4], M. N. SHADLEN[5];
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Abstract: To make decisions, animals not only need to accumulate evidence for different options, they must also terminate this accumulation process to commit to a choice. While the computational and circuit mechanisms underlying evidence accumulation are well studied, those

underlying decision termination are largely unknown. Recent evidence from nonhuman primates confirms a theoretical model prediction that the superior colliculus (SC) facilitates termination via a large burst of activity when cortical evidence accumulation reaches a threshold, initiating a saccadic choice. However, existing threshold detection models of SC fail to explain the single trial bursting dynamics observed in SC neural recordings, which include smaller non-saccadic bursts throughout the evidence accumulation process. This discrepancy necessitates new models to reveal the mechanisms underlying the SC's rich dynamics and its interactions with other regions of the decision-making circuit. To address this gap, we built a rate-based model of SC neural activity during a reaction-time motion discrimination perceptual choice task. We found that both decision-terminating and non-saccadic burst dynamics were well-captured by a model of SC that filters the evidence integration signal from the lateral intraparietal area (LIP) and performs threshold detection on the filtered signal, but not by a model relying on threshold detection alone. Critically, only filters that accounted for a mixture of two features of the LIP signal - both its magnitude and derivative - captured single trial SC burst dynamics when we fit the model to nonhuman primate neural data. This indicates that SC computations leverage both the degree of evidence and momentary changes in evidence during decision making. We further demonstrated that such temporal filtering could be implemented by biologically realistic mechanisms, including disynaptic inhibition and spike rate adaptation. Finally, we embedded these computations into a larger closed-loop circuit, where the LIP accumulates evidence, the SC temporally filters LIP activity and thresholds the filtered activity to terminate decisions, the basal ganglia set the decision termination threshold, and a site such as the pulvinar nucleus relays the termination signal to end the integration process in LIP. Together, this work models the dynamics governing LIP-SC interactions for decision termination, calls for future experiments to test the hypothesized circuit, and highlights the role of temporal filtering in cognitive computations.

Disclosures: L. Brown: None. **A.E. Comrie:** None. **G.M. Stine:** None. **M.S. Goldman:** None. **M.N. Shadlen:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.06/GG11

Topic: H.04. Emotion

Support: 1R01MH127214
A Vallee Scholars Award

Title: Effect of early life stress on microglia function in developing nucleus accumbens

Authors: *C. CHRISTENSEN[1], S. V. BLAGBURN-BLANCO[2], J. M. BLAGBURN[3], L. A. DENARDO[2];
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Abstract: Early life adversity -such as poverty and neglect- occurring during key developmental periods, is a major risk factor for developing mental health disorders (ELA). These adverse events alter the maturation of reward and threat-related brain regions, such as the nucleus accumbens (NAc) and the medial prefrontal cortex (mPFC), leading to dysfunctional activity in this region and maladaptive behaviors. This project examines how ELA impacts microglia, stress-sensitive immune cells, which shape developing neural circuits through synapse surveillance and by mediating synaptogenesis and synapse pruning. We use an established ELA model in mice, known as the limited bedding and nesting (LBN) protocol. This approach stresses the dams by giving them access to sparse bedding resources, and in turn stresses their pups. Our findings show elevated microglial densities in the NAcs of LBN juvenile mice compared to controls. In mPFC, we also find increased microglial densities in the juvenile timepoint. This region is involved in the reward circuit, specifically in reward processing and responding, and projects to the NAc. This suggests an effect of LBN on multiple regions involved in the reward circuit. We find that the NAc as a whole has increased Iba1 staining at infant and juvenile time points, indicating an increase in microglial branching. In line with this, we find an increased microglial branching complexity that persists through adolescence. This suggests that ELA alters microglial phenotypes during development, potentially disrupting their normal circuit refinement activity within the NAc, and leading to long term behavioral changes. Through behavioral assays, we find increased anxiety like behaviors in adult LBN males. This project advances our understanding of the impacts of stress on microglial complexity and activity, as well as long term behavioral changes.

Disclosures: C. Christensen: None. **S.V. Blagburn-Blanco:** None. **J.M. Blagburn:** None. **L.A. DeNardo:** None.

Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

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Program #/Poster #: PSTR405.07/GG12

Topic: H.04. Emotion

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Whitehall Research Grant

Title: A cell type-specific mechanism driving the rapid antidepressant effects of transcranial magnetic stimulation

Authors: *M. GONGWER[1], A. QI[1], A. ENOS[1], T. SOLAKOGLU[1], C. KLUNE[1], R. AHMED[1], A. Q. KASHAY[1], Y. YANG[4], H. LU[5], A. LEUCHTER[6], L. A. DENARDO[2], S. WILKE[3]; [2]Physiol., [3]Psychiatry and Biobehavioral Sci., [1]UCLA, Los Angeles, CA; [4]Natl. Inst. of Drug Abuse, Baltimore, MD; [5]Natl. Inst. on Drug Abuse, Natl. Inst. on Drug Abuse, NIH, Baltimore, MD; [6]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive method for brain stimulation that is an emerging treatment for depression and other neuropsychiatric disorders. Clinical rTMS is proposed to exert therapeutic effects by driving neuroplastic changes in cortical networks. However, the underlying circuit mechanisms are poorly understood, limiting the rational design of more precise and effective treatment protocols. Here, we present a novel preclinical model of rTMS, utilizing the first rodent coil capable of both highly focal (<2mm) and suprathreshold stimulation of a cortical subregion. By combining medial prefrontal cortex (mPFC) targeted clinical rTMS with chronic stress, fiber photometry and chemogenetics, we reveal a cell type-specific mechanism by which rTMS may drive therapeutic effects in depression. We show that accelerated intermittent theta-burst stimulation (iTBS) over mPFC can reverse behavioral deficits associated with chronic stress. Using fiber photometry, we recorded neural dynamics from cortex-projecting intratelencephalic (IT) neurons and subcortical-projecting pyramidal tract (PT) neurons in mPFC during and after accelerated iTBS. Both cell types responded to individual iTBS trains with an acute increase in activity. However, across a 10-minute iTBS session, IT neurons exhibited increasing activity over time, while PT neurons displayed a trend of decreased activity. One day later, iTBS-treated animals displayed enhanced IT neuron activation during effortful responses to an inescapable stressor, but no changes were observed in the activity of PT neurons. In line with this, high-resolution confocal imaging revealed that aiTBS reversed stress-related loss of dendritic spines on IT, but not PT neurons, further demonstrating cell type-specific effects of stimulation. Finally, chemogenetic suppression of IT, but not PT neuron activity during accelerated iTBS was sufficient to block the antidepressant-like effects of stimulation. Together, these results reveal a critical role for cell-type specific plasticity in the therapeutic effects of rTMS.

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Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

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Program #/Poster #: PSTR405.08/GG13

Topic: H.04. Emotion

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Title: Cell type-specific modulation of prefrontal inhibition underlying the rapid antidepressant effects of transcranial magnetic stimulation

Authors: *R. AHMED[1], M. GONGWER[2], A. QI[1], J. BARRY[1], T. SOLAKOGLU[1], Y. YANG[6], H. LU[7], M. S. LEVINE[3], C. CEPEDA[4], A. LEUCHTER[8], L. A. DENARDO[2], S. WILKE[5]; [2]Physiol., [3]Semel Inst., [4]IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI., [5]Psychiatry and Biobehavioral Sci., [1]UCLA, Los Angeles, CA; [6]Natl. Inst. of Drug Abuse, Baltimore, MD; [7]Natl. Inst. on Drug Abuse, Natl. Inst. on Drug Abuse, NIH, Baltimore, MD; [8]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Repetitive transcranial magnetic stimulation (rTMS) is a form of therapeutic brain stimulation that drives lasting changes in brain function. When targeted to the prefrontal cortex (PFC), rTMS can effectively treat depression and other neuropsychiatric disorders. However, the neural mechanisms underlying its therapeutic effects are poorly understood. To address this gap, we developed and validated a novel mouse model of rTMS with superior clinical face validity. Accelerated intermittent theta burst stimulation (aiTBS) - the first rapid-acting rTMS antidepressant protocol - targeting the dorsomedial PFC (dmPFC) rapidly reversed stress-induced deficits in several depression-related assays, supporting the validity of our model. iTBS pulse patterns are thought to promote increased cortical excitability by promoting long-term potentiation of excitatory synapses onto pyramidal neurons. However, recent work has proposed an alternative model in which decreased intracortical inhibition promotes increased excitability. Here, we investigated the antidepressant effects of aiTBS using in vivo photometry recordings, slice electrophysiology, and chemogenetic inhibition in three classes of prefrontal inhibitory interneurons: those expressing parvalbumin (PV), somatostatin (SST) and vasoactive intestinal polypeptide (VIP).
Fiber photometry recordings revealed that aiTBS increased the activity of SST neurons, but suppressed the activity of PV and VIP neurons in the context of depression-related behavior. Slice electrophysiology recordings revealed enhanced inhibitory synaptic input to PV neurons from SST neurons, suggesting a disinhibitory mechanism may mediate changes in prefrontal excitability. Finally, we found that chemogenetic suppression of SST neuron activity during aiTBS blocks its antidepressant-like behavioral effects. This demonstrates that aiTBS driven activation of SST interneurons is necessary for therapeutic behavioral effect. Together, these findings support a model in which specific subtypes of inhibitory interneurons play unique roles in the therapeutic effects of aiTBS.

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Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

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Program #/Poster #: PSTR405.09/GG14

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NIH NCATS KL2TR002381
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Title: Objective, AI-derived brain biomarkers reliably track treatment response in SCC-DBS for treatment-resistant depression

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[1]Electrical and Computer Engin., Georgia Inst. of Technol., Atlanta, GA; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Psychiatry and Behavioral Sci., Emory Univ., Atlanta, GA

Abstract: Deep brain stimulation (DBS) targeting the subcallosal cingulate cortex (SCC) has shown promise for treating treatment-resistant depression (TRD). However, conventional assessments of depression severity rely heavily on subjective evaluations, often influenced by external factors. This limitation emphasizes the critical need for objective, brain-based biomarkers to guide clinical decisions and treatment monitoring. Previously, our group developed a biomarker (SDC) from local field potentials (LFP) using explainable artificial intelligence (xAI) techniques, successfully indicating treatment response states in six TRD patients undergoing SCC-DBS therapy. In the current study, we replicated this biomarker in a new cohort of seven participants. We utilized a new chronic monitoring platform designed to record LFP data at higher frequency, allowing for daily-scale assessments. Biomarker performance was evaluated under various stimulation conditions (DBS on vs. off) and at different times throughout the day, enabling examination of possible circadian influences. The biomarker replication demonstrated robust accuracy in the new patient cohort when compared with HDRS-17 (accuracy: 0.72 ± 0.2; original cohort: 0.85 ± 0.1), confirming its reliability and consistent performance under different recording conditions. Further analyses revealed narrowband spectral power surrogates as simplified markers at distinct frequency bands: 12-17 Hz in the left and 10-15 Hz in the right hemisphere. These simplified biomarkers reliably tracked depressive states across both patient cohorts (SDC accuracy: 0.87 ± 0.2 Left, 0.88 ± 0.1 Right; HDRS-17 accuracy: 0.69 ± 0.3 Left, 0.79 ± 0.2 Right). Notably, hemispheric differences in these markers were observed among participants, reflecting variations in brain synchrony possibly due to structural and functional differences within the cingulum bundle (CB) and midcingulate cortex (MCC). Participants showing synchronized hemispheric biomarker changes demonstrated significantly higher fractional anisotropy (FA) within the CB, indicating a potential link between structural connectivity and synchronized hemispheric engagement. Our findings support the feasibility and reliability of brain-based biomarkers for objectively tracking depression recovery,

highlight simplified markers suitable for clinical application, and that can highlight individual differences influencing treatment response. These findings may facilitate broader clinical implementation of DBS therapy for depression and may accelerate optimization of personalized DBS therapy for depression.

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Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR405.10/GG15

Topic: I.03. Decision Making

Support: IK2 CX001584
1I01CX002456

Title: Neuroanatomical markers of hedonic tone and reward-based decision-making in trauma-exposed Veterans

Authors: *E. MEHTA[1], R. ALJENABI[1], S. NORTHUP[1], J. LEE[1], S. NORMAN[3], A. D. SPADONI[4], A. SIMMONS[4], K. M. HARLE[2];
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Abstract: Trauma-exposed Veterans commonly experience dysregulated reward processing that manifests as reduced hedonic tone, i.e., anhedonia. Anhedonia has been linked to structural and functional differences in brain regions involved in affect and reward processing, particularly the caudate and dorsolateral prefrontal cortex (DLPFC). The caudate plays a role in goal-directed behavior and learning paradigms, while the DLPFC contributes to the decision-making process. However, it remains unclear whether anhedonia-related anatomical abnormalities impact reward processing mechanisms, particularly in trauma-exposed individuals. We investigated this question with a reinforcement learning modeling approach. Trauma-exposed Veterans (N = 57) completed a 3-arm monetary bandit task where they selected between different slot machines to maximize overall reward. Using hierarchical Bayesian estimation, we fit a Rescorla-Wagner

model to participants' choices to derive parameters of reward-based decision-making, including initial beliefs about reward probabilities, learning rate, and degree of reward maximization. Regional brain volumes for the caudate and DLPFC were extracted with FreeSurfer. Participants completed the positive subscale of the Positive and Negative Affect Schedule (PANAS) to assess hedonic capacity. Regression and mediation analyses with bootstrapping were conducted to determine the effect of region volumes on the relationship between hedonic tone and reward parameters. After controlling for age, the right caudate volume significantly mediated the relationship between hedonic tone and reward maximization ($\beta = 0.031$, 95% CI = [0.0033, 0.07] $p = .026$), suggesting that greater hedonic tone was associated with smaller caudate volume, which then predicted higher reward maximization. The total effect was not significant, suggesting a full indirect effect. While a similar pattern was observed for the left caudate volume, it did not survive a Bonferroni comparison for multiple tests. Additionally, a linear regression showed that larger left DLPFC volume significantly predicted, but did not mediate, higher initial reward expectations ($\beta = .000073$, $p = .032$), after controlling for age, suggesting the DLPFC may influence early-stage beliefs in reward learning. These findings suggest that anatomical differences in reward processing regions, such as the right caudate and left DLPFC, may contribute to individual mechanisms of reward exploration and their modulation by hedonic tone. Understanding the neural substrates of hedonic tone may help inform precision treatments to improve reward processing in trauma-exposed populations.

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Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

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Program #/Poster #: PSTR405.11/HH1

Topic: H.04. Emotion

Support: National Institute of Mental Health R01MH098348

Title: Frontoparietal Network Connectivity, Mental Health, and Family Connectedness

Authors: *O. IOSIPCHUK[1], A. BOLARAM[1], D. H. SCHULTZ[2], H. E. DARK[1], S. MRUG[1], D. C. KNIGHT[1];
[1]Psychology, Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Nebraska, Lincoln, Lincoln, NE

Abstract: The frontoparietal network (FPN) has been linked to depression, anxiety, and Posttraumatic Stress Disorder (PTSD). The dysfunction of this network may disrupt emotion regulation processes, which may increase susceptibility to mental health disorders. The FPN provides top-down control over the functions of other brain regions that support emotional expression. Further, FPN connectivity with other functional networks (e.g., limbic network) may

underlie the expression of mental health symptoms. The relationship between the FPN and mental health symptoms is affected by a number of complex socioenvironmental factors. For example, social connectedness supports resilience to mental health disorders and may also affect the connectivity of the FPN. Therefore, the relationship between FPN connectivity, mental health symptoms, and family connectedness is an important area of study. The present study examined the interrelationships between FPN connectivity, mental health, and family connectedness. We hypothesized that greater family connectedness would be associated with lower mental health symptoms. Further, we expected that lower symptoms of depression, anxiety, and PTSD would be associated with higher FPN between-network connectivity. We also hypothesized that individuals with higher family connectedness would exhibit greater FPN between-network connectivity, and that family connectedness would moderate the relationship between the FPN between-network connectivity and mental health symptoms. Resting-state functional magnetic resonance imaging data were collected from 287 adults. Between-network global connectivity (BGC) was used to calculate the functional connectivity between the FPN and brain regions in other functional networks. Mental health symptoms and family connectedness were assessed via questionnaires. We found significant negative relationships between family connectedness and depression ($p = .004$), anxiety ($p = .02$), and PTSD ($p < .001$) symptoms. There were no relationships observed between FPN BGC and depression, anxiety, and PTSD symptoms ($p > .05$). However, there was a significant negative relationship between FPN BGC and family connectedness ($p = .036$). However, family connectedness did not moderate the relationship between the FPN BGC and depression, anxiety, and PTSD symptoms ($p > .05$). The present findings suggest that individuals with higher family connectedness experience fewer mental health symptoms and may rely less on the FPN to regulate emotional responses. These findings underscore the importance of social connectedness (i.e., family connectedness) in the expression of mental health symptoms.

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Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

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Program #/Poster #: PSTR405.12/HH2

Topic: H.04. Emotion

Support: Philanthropic funding

Title: Exploring the therapeutic potential of psilocybin for Obsessive-Compulsive Disorder - A translational approach

Authors: *P. TIWARI[1], B. W. JENKINS[3], C. F. MOORE[2], D. YADEN[1], E. M. WEERTS[1]; [2]Psychiatry and Behavioral Sci., [1]Johns Hopkins Univ., Baltimore, MD; [3]Johns Hopkins Med. Institutions, Baltimore, MD

Abstract: Objective: Obsessive-compulsive disorder (OCD) is a common and debilitating psychiatric condition. Current treatment options (primarily SSRIs) offer limited benefit for many individuals, leaving a significant portion of patients inadequately treated. This ongoing study is investigating the possible therapeutic effects of psilocybin using a neurodevelopmental rat model of OCD-like behavior, that parallels our Phase I clinical trial examining the impact of two psilocybin dosing sessions in individuals with OCD. Our preclinical model will evaluate psilocybin's effects in a disease-relevant context, allowing for a deeper understanding of the underlying neurobiological mechanisms of psilocybin's therapeutic effects.
Methods: To generate a developmental model of OCD, Sprague-Dawley rat pups were administered clomipramine or saline control from postnatal days 9 to 16. We assessed for OCD-like behaviors in adults, including compulsive burying in the marble burying test (MBT), facial grooming, and circling, as well as comorbid anxiety-related measures using conflict anxiety mazes like the open field test (OFT) and elevated plus maze (EPM). To identify the most effective dose of psilocybin on these behaviors, naïve adult rats were administered psilocybin (0, 0.5, 1, or 1.5 mg/kg) to determine the acute and post-acute (4h) behavioral response in these same assays. We will use the selected dose to then administer psilocybin on two alternate days in the rat model (paralleling the Phase I clinical trial protocol) to assess both acute and persistent effects on OCD-like behaviors. Brains will be perfused and processed using immunohistochemistry for c-Fos activation and other cellular markers (e.g. plasticity markers) in brain areas implicated in OCD (e.g., basal ganglia).
Results: Our animal model of OCD-like behavior resulted in increased burying response, validating our approach. Findings in naïve animals revealed a distinct, dose-dependent acute behavioral response to psilocybin administration, as seen with head-twitch response, facial grooming, total locomotion, and compulsive behavior on the MBT. We also evaluated post-acute effects of psilocybin on compulsive and anxiety-related behaviors. Ongoing studies aim to elucidate the underlying neurobiological mechanisms through cellular and molecular analyses of activity patterns and neuroplasticity.
Conclusion: This study employs a translational approach to evaluate the therapeutic potential of psilocybin in OCD aiming to improve understanding of psilocybin's effects and inform future research directions in the treatment of OCD.

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Poster

PSTR405: Neural Mechanisms and Correlates of Psychopathologies and Addiction

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Topic: H.04. Emotion

Support: NIMH 1R01MH127214-01A1
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NIGMS T32 GM008042
NIGMS T32 GM152342

Title: Impacts of early life adversity on microglial function, nucleus accumbens activity and avoidance behaviors throughout development

Authors: *S. V. BLAGBURN-BLANCO[1], C. CHRISTENSEN[1], J. M. BLAGBURN[3], L. A. DENARDO[2];
[2]Physiol., [1]UCLA, Los Angeles, CA; [3]Inst. Neurobiol, Univ. Puerto Rico, San Juan, PR

Abstract: Early life adversity (ELA) is a major risk factor for developing mood and anxiety disorders in childhood and later in life. Studies in humans and animal models suggest that ELA disrupts the development of key emotion centers in the brain, including the nucleus accumbens (NAc), leading to threat-sensitization, dysfunctional reward processing, and maladaptive avoidance later in life. However, most preclinical studies of ELA examine the behavioral and biological consequences of early life stress in adult animals. As such, the mechanisms through which ELA impacts developing cells and circuits in the NAc, altering its function and conferring a long-lasting susceptibility to psychiatric disease, remain poorly understood. In this study, we use a limited bedding and nesting (LBN) mouse model of early life adversity, in combination with immunohistochemistry and viral labeling techniques to examine how ELA impacts cells within the NAc at timepoints spanning from postnatal periods to adulthood (P12-P70). We focus on microglia, the brain-resident immune cells. Microglia are responsive to stress and play crucial roles in synaptogenesis and synapse pruning. We hypothesized that ELA in the form of resource scarcity and caregiver stress (LBN) alters developmental microglial phenotypes and circuit-refinement activity in the NAc, resulting in maladaptive circuit maturation that underlies ELA-induced behavioral dysfunction. We discovered a transient, significant increase in microglial density in the NAcs of juvenile LBN mice compared to age-matched, standard-reared (SR) mice. We also observed that NAc microglia were more ramified in LBN mice than in SR controls. Further, preliminary analyses of dendritic spines on NAc medium spiny neurons reveal an age-dependent effect of rearing condition on dendritic spine densities. Ongoing analyses will determine whether these increases in microglial densities and ramification are associated with changes to microglial lysosomes and engulfment of synaptic material. In addition to these microglial and neuronal changes in the NAc, we found that LBN mice exhibit enhanced avoidance behaviors in an age- and sex-dependent manner. Overall, this project will reveal the neurobiological mechanisms through which ELA impacts developing neural circuits and alters avoidance behaviors.

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Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.01/HH4

Topic: H.04. Emotion

Title: Individual differences in activity level and risk assessment in the Madagascar hissing cockroach, Gromphadorhina portentosa

Authors: S. JOHNSON, **A. VILLALON**, C. BRACKEN, E. SHOWGREN, *J. O. TAYLOR; Utah Valley Univ., Orem, UT

Abstract: Madagascar hissing cockroaches (MHC) are invertebrate insects that are inexpensive, hardy, and easy to maintain even in small spaces. Due to this, they provide an excellent animal model for simple behavioral tasks frequently used to explore learning and risk assessment behavior. Previous work in the lab has shown that large variance in activity levels appear to be a consistent individual difference that can be problematic due to floor or ceiling effects in behavioral assessments. The current studies were conducted to explore the relationship between activity level and risk-assessment behavior with both males and females. Subjects were observed in single-session exploration of a custom open field apparatus. Video data was coded to determine activity levels as well as risk assessment behavior. Consistent with previous work in our lab, MHC subjects demonstrated high levels of individual differences in activity. Measures of central tendency in activity for were used as an initial separation into high activity (HA), low activity (LA) subjects. Subjects classified by median split revealed differences in patterns of activity throughout the session in addition to overall activity, with the HA cluster consistently moving throughout, whereas the LA were initially as active, but reduced activity over a short period of time. Interestingly, despite the significant differences in movement, there were no significant differences in risk assessment measures on the open field. Additional pilot observations using behavioral pharmacology, primarily with ethanol administration further support at least a partial dissociation between activity and risk-assessment related behaviors with the MHC. These results indicate that screening for activity level will allow us to use HA roaches to mitigate floor effects with assays for learning and risk-assessment behavior.

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Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.02/HH5

Topic: H.05. Mood Disorders

Support: Department of Pharmacology, Toxicology & Neuroscience

Title: Stress, signaling, and behavior: A multimodal assessment in wildtype and GR-deficient zebrafish

Authors: *A. THOMAS;
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Abstract: Dysregulation of stress feedback signaling can lead to long-term adverse outcomes, including heightened susceptibility to anxiety and depression. Prior studies in humans and rodents have shown that stressful stimuli can impair this regulatory system. This study used adult wildtype (WT) and glucocorticoid receptor ENU knockout (GRKO) zebrafish to examine the delayed behavioral effects of acute and moderate stress, as well as the impact of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) antagonists. GRKO zebrafish exhibit disrupted GR feedback signaling, providing a model for evaluating the behavioral consequences of impaired glucocorticoid regulation. We hypothesized that GR dysfunction, whether genetic or pharmacological, alters stress responses and promotes anxiety- and depression-like behaviors in adult zebrafish, with GRKO fish showing baseline anxiety and distinct behavioral and cortisol profiles compared to wildtypes. Zebrafish were exposed to either a 90-minute acute severe stressor or a three-day moderate stress protocol, with or without pretreatment using the GR antagonist mifepristone or the MR antagonist spironolactone. Behavioral tests, including the open field test, novel tank test, zebrafish tail immobilization assay, and compartment preference test, were conducted one week later to assess anxiety-like behavior, exploratory activity, and despair-like responses. Whole-body cortisol was also measured. At baseline, GRKOs exhibited increased anxiety-like behavior compared to WT fish. Following moderate stress, WT fish showed heightened anxiety-like responses, while GRKOs did not. WT fish also displayed cortisol suppression post-testing, a physiological response absent in GRKOs. Acute stress alone did not induce long-term behavioral changes. Moderate stress did not significantly affect locomotion or despair-like behavior in either genotype. Mifepristone produced dose-dependent behavioral effects. A low dose combined with acute stress elicited anxiolytic-like behavior, while a moderate dose reduced anxiety-like behavior even without stress exposure. GR or MR antagonism combined with moderate stress did not affect locomotion or despair-like behavior but altered exploratory and anxiety-related outcomes, particularly in mifepristone-treated fish. These findings highlight the role of GR signaling in shaping stress-related behavior and physiology and support GRKO zebrafish as a model for dysregulated stress responses.

Disclosures: A. Thomas: None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.03/HH6

Topic: H.04. Emotion

Support: LSU Health Shreveport Department of Pharmacology, Toxicology, and Neuroscience

Title: Kisspeptin1 mutation decreases fear-related and increases anxiety-related behaviors in zebrafish

Authors: *T. AJAYI, C. D. SCHMOUTZ;
Pharmacology, Toxicology, and Neurosci., LSU Hlth. Shreveport, Shreveport, LA

Abstract: Kisspeptin1 (Kiss1) is a signaling neuropeptide in the hypothalamic-pituitary-gonadal axis regulating the downstream release of reproductive hormones. While Kiss1-expressing neurons are widespread in the hypothalamus and linked to reproductive functions in mammals, its localization and functions in rodents and zebrafish have been identified in additional locations that regulate mood states and their associated behavioral responses. In mice and zebrafish models, administration of a Kiss1 agonist results in significantly decreased anxiety. Moreover, ablation of neurons expressing the Kiss1 receptor reduces fear response to alarm substance (fear-inducing stimuli) in zebrafish. However, there is still limited knowledge on the mechanisms behind the behaviors responsible for these effects and how additional manipulations of the Kiss1 ligand-receptor system can alter fear- and anxiety-related behaviors. We hypothesized that mutation of the Kiss1 neuropeptide gene in wildtype (strain AB) zebrafish will decrease fear-related behaviors and increase anxiety-related behaviors. To test this, we used a Kiss1 mutant zebrafish model (kiss1^stl182/stl182) and characterized fear- and anxiety-related behaviors using neurobehavioral assays: open field test, novel tank diving task, and alarm substance exposure. Following alarm substance exposure, kiss1^stl182/stl182 fish had significantly decreased fear-related responses. Additionally, kiss1^stl182/stl182 zebrafish displayed significantly increased anxiety-related responses in the open field test and novel tank diving task. These results suggest that mutation of the Kiss1 gene can alter fear- and anxiety-related responses in zebrafish. Ongoing studies in Kiss1 mutant fish will further characterize anxiety-related phenotypes, elucidate the generalizability of the disrupted fear response to other fear-inducing stimuli, and pharmacologically rescue altered fear- and anxiety-related responses to understand underlying mechanisms in Kiss1 signaling.

Disclosures: T. Ajayi: None. **C.D. Schmoutz:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Program #/Poster #: PSTR406.04/HH7

Topic: H.04. Emotion

Title: Psilocybin and the related tryptamine, norbaeocystin, exert acute anxiogenic effects that depend on sex

Authors: *M. J. SAVINI[1], K. M. KING[1], N. M. MOLNAR[1], E. J. SCHNEIDER[1], S. E. STRAUB[1], J. PARKER[1], L. P. PUPPEL[1], A. K. AL-OLIMAT[1], A. M. BACH[1], A. JONES[2], P. R. ZOLADZ[1];

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Abstract: Single treatments with psychedelics, such as psilocybin, have demonstrated rapid and long-lasting antidepressant effects in both preclinical and clinical studies. However, psychedelics often produce unfavorable psychoactive effects that could worsen existing psychological distress in individuals with depression and anxiety. Thus, there is interest in identifying compounds that are similar to psychedelics but do not produce such adverse side effects. Several psilocybin-related tryptamines (e.g., norbaeocystin) that do not produce psychoactive effects can be found in "magic" mushrooms, and previous work has shown that some of these compounds induce antidepressant effects in preclinical models. Less is known about the acute impact of these agents on anxiety-like behavior, and because psychedelics can exert acute anxiogenic effects, it is important to determine whether related compounds do the same. Thus, we compared the effects of psilocybin and norbaeocystin on anxiety-like behavior in male and female rats. In the first experiment, adult male and female Sprague-Dawley rats were treated with 1 mg/kg psilocybin (PSIL), 1 mg/kg norbaeocystin (NOR), or vehicle 30 min prior to a 5-min trial on an elevated plus maze (EPM) or in an open field test (OFT). On the EPM, PSIL led to decreased open arm time and fewer overall arm entries in both males and females. NOR led to decreased open arm time in males, while having no significant impact on female behavior. Findings for the OFT mirrored those from the EPM. PSIL led to decreased time in the center of the open field in males and decreased distance traveled in both males and females. NOR led to decreased time in the center of the open field in males, while having no significant impact on female behavior. In the second experiment, we assessed whether the anxiogenic effects of PSIL and NOR would persist for 24 hr. Rats were administered PSIL, NOR, or vehicle and tested on the EPM 24 hr later. The results revealed no significant effects of PSIL or NOR on EPM behavior. Overall, our results indicate that PSIL exerts acute anxiogenic effects in both males and females, while NOR selectively increases anxiety-like behavior in males. These sex differences warrant further research and may be an important consideration in identifying which psychedelic-like compounds are most effective in treating psychological conditions in both sexes.

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Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.05/HH8

Topic: H.04. Emotion

Support: Natural Science Foundation of Beijing Municipality 5244028
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CIBR start-up

Title: Threats and Allies: Social Modulation of Innate Defensive Behaviors

Authors: X. GAO[1], L.-Y. LI[2], *Y.-T. LI[3];
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Abstract: In group-living animals, defensive responses to predators are shaped by social context. While extensive research has examined social modulation of learned fear responses, these effects—and the underlying neural circuits—may differ from those governing innate fear. Recent studies using innate fear paradigms have begun to uncover how social cues influence defensive behaviors across species, yet it remains unclear how social hierarchy modulates these innate responses, and whether this modulation depends on the nature of the threat. To address these questions, we investigated the social modulation of innate fear in mice under two ecologically relevant threat conditions: a transient visual looming stimulus and a sustained live rat. Pair-housed mice with established dominance ranks were exposed to these threats either alone or with their social partner. Behavioral responses were quantitatively analyzed to determine the effects of social context. We found that social presence generally alleviates threat-induced stress across both social ranks and threat types, while modulating defensive behaviors in a rank- and threat-specific manner. During looming exposure, dominant mice exhibit greater benefits from social presence, characterized by weaker defensive responses and a reduction in defensive time. In contrast, during rat exposure, social context promotes a shift from passive to active defenses, particularly in dominant individuals. Furthermore, threat exposure enhances social behaviors in a rank-specific manner, suggesting a bidirectional relationship between social and defensive behaviors. Together, these findings demonstrate that hierarchical social context plays a critical role in shaping innate defensive behaviors, and that threat exposure reciprocally influences social behavior. This work provides a framework for probing the neural basis of socially modulated innate fear.

Disclosures: X. Gao: None. **L. Li:** None. **Y. Li:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Program #/Poster #: PSTR406.06/HH9

Topic: H.04. Emotion

Support: NIH NS119783

Title: Optogenetic stimulation of Purkinje cells in the cerebellar vermis results in robust real-time place aversion

Authors: *R. A. MCGANN[1], I. M. RAMAN[2], C. E. VAAGA[1];
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Abstract: The cerebellum has classically been viewed as a motor structure, however, there is increasing appreciation for its non-motor functions. For example, in humans, cerebellar hypoplasia or damage results in a constellation of cognitive and affective dysfunctions, termed Cerebellar Cognitive Affective Syndrome. The cerebellar contribution to affective regulation, especially fear behaviors, likely involves broad projections to numerous limbic and autonomic brain regions, however, the precise role of the cerebellum in modulating affective and innate fear remains unresolved. In the present study, we assess how direct manipulation of cortical cerebellar activity alters affective behavior and response to innate predator threats. First, in transgenic mice that express ChR2 under control of the L7 promotor, we demonstrate that 100 Hz optogenetic stimulation of vermal Purkinje (vPkj) cells significantly reduces the firing rate of neurons in the medial (fastigial) cerebellar nucleus. We then used this technique to evaluate how disruption of cerebellar output influences both affective disposition and defensive response to innate predator threats. In an open-field chamber, unexpected vPkj cell stimulation significantly increased anxiety-like behaviors. Additionally, in a real-time place preference assay, we found that vPkj cell stimulation was highly aversive, resistant to reversal, and persisted for multiple days. Together this data suggests that stimulation of the cerebellar vermis is, in itself, highly aversive. Furthermore, when a single epoch of vPkj stimulation was paired with looming predator threats, we observed a disruption in innate freezing behavior and the adaptive habituation pattern seen in wildtype mice following repeated exposures. This suggests intact cerebellar activity is acutely necessary for appropriate fear appraisal. Future work now focuses on evaluating the extent to which vPkj cell stimulation mediates these aversive effects through the regulation of autonomic or affective circuits. Collectively, this work provides strong evidence of the cerebellum's pivotal, yet under-appreciated, role in emotional and autonomic integration, processing, and modulation.

Disclosures: R.A. McGann: None. **I.M. Raman:** None. **C.E. Vaaga:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Program #/Poster #: PSTR406.07/HH10

Topic: H.04. Emotion

Title: Plcb1 regulates observational fear via mediating myelination in acc-bla circuits

Authors: *S. LEE[1,2], B. CHANG[3], B. LEE[4], C. LEE[5], H.-S. SHIN[6];
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for Cognition and Sociality, [4]Inst. for Basic Sci., Daejeon, Korea, Republic of; [6]Ctr. for Cognition and Sociality, Inst. for Basic Sci., Seoul, Korea, Republic of

Abstract: Myelination is known to be enhanced by neuronal activities and social experiences. It is involved in regulating brain oscillations which are critical for behavior and cognitive function. We found that phospholipase C beta 1(PLCb1) knockout mice exhibit impaired observational fear, a basic form of emphatic behavior. Furthermore, demyelination was detected in the anterior cingulate cortex (ACC) and corpus callosum of this knockout mouse. Conditional knockout of PLCb1 in the ACC-basolateral amygdala (BLA) circuits decreased observational fear. We detect that the axon of BLA projecting ACC neurons pass through the cingulum and corpus callosum, surrounding the white matter tract of the ACC. In addition, Knockdown of myelin basic protein (MBP) in the ACC disrupted theta oscillation and reduced observational fear. These results suggest that PLCb1 regulates observational fear by modulating theta oscillations through myelination.

Disclosures: S. lee: None. **B. Chang:** None. **B. Lee:** None. **C. Lee:** None. **H. Shin:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Program #/Poster #: PSTR406.08/HH11

Topic: H.04. Emotion

Title: Probiotic ASVL002 alleviates depression-like behaviors induced by repeated social defeat stress

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Abstract: Major depressive disorder (MDD) is a common and disabling psychiatric condition characterized by persistent negative affect and emotional dysregulation. Although fast-acting antidepressants such as ketamine have shown efficacy in alleviating depressive symptoms, their effects are often limited, and not all patients achieve remission. There is growing interest in gut microbiota-targeted interventions as alternative or adjunctive treatments for mood disorders.In this study, we employed the repeated social defeat stress (RSDS) model to induce robust depressive-like behaviors in mice, including social avoidance and anhedonia. A single intraperitoneal injection of ketamine (10 mg/kg) effectively reversed the affective symptoms associated with RSDS exposure. To explore microbiota-based strategies, we administered a specific probiotic strain, ASVL002, to stressed mice. Remarkably, ASVL002 treatment significantly alleviated depressive-like behaviors, showing comparable or even superior efficacy to ketamine in social interaction and sucrose preference tests.These findings suggest that ASVL002 may serve as a promising microbiota-based therapeutic for stress-induced depression.

Future studies will focus on elucidating the underlying mechanisms by which ASVL002 modulates emotional behavior, including microbiota-derived metabolite signaling relevant to emotional regulation.

Disclosures: H. Tung: None. **P. Wang:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Title: Oxytocin's Differential Modulation of Hyperalgesic Priming and Pain Neuroplasticity in Male and Female Mice

Authors: *D. B. SOUZA[1], N. UREL-CARNEIRO[1], A. BRAGA DIAS[1], R. ALVARENGA[1], R. L. NUNES-DE-SOUZA[2];
[1]UNESP, Araraquara, Brazil; [2]Pharmacol., Sao Paulo State Univ., Araraquara, Brazil

Abstract: Sex differences in pain are widely recognized, arising from genetic, neurochemical, hormonal, and circuit-level distinctions between males and females. Neuropeptide oxytocin emerges as a key modulator, with evidence indicating its analgesic effects. This study investigated sex-specific oxytocin modulation of pain in male and female mice subjected to hyperalgesic priming. Our approach involved three experiments: assessing plasma oxytocin levels, evaluating the effects of intraperitoneal oxytocin administration (1 and 5 mg/kg), and mapping projections from the paraventricular hypothalamic nucleus (PVN), the primary source of oxytocin, to various brain regions. In Experiment 1, hyperalgesic priming significantly decreased plasma oxytocin levels in both sexes ($F(1,18)=12.19, p=0.002$). In Experiment 2, a significant time x treatment interaction ($F(6,32)=3.02, p<0.05$) was observed exclusively in females. Specifically, female mice treated with 5 mg/kg oxytocin post-incision showed reduced mechanical hyperalgesia 3 hours after PGE2 administration compared to the saline group. Additionally, female mice receiving 1 mg/kg oxytocin at both time points exhibited reduced mechanical hyperalgesia 24 hours after PGE2. Preliminary results from Experiment 3 confirmed PVN projections to the amygdaloid nucleus, periaqueductal gray matter, and rostroventral nucleus. These findings suggest that oxytocin may exert analgesic effects on mechanical hyperalgesia in a sexually dimorphic manner in mice subjected to the hyperalgesic priming model. The study was conducted following the ARRIVE guideline 2, and was approved by the local Research Ethics Committee (CEUA/FCF/CAr 19/2022).

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Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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General Program of Natural Science Foundation of Guangdong Province, China (2025A1515011068)

Title: Reduced Oxytocin Signaling in the dBNST Drives the Transition from Acute Pain to Persistent Anxiety

Authors: *Y. XIAODAN[1], S. FANG[2], L. HUANG[3], B. LI[4];
[1]SUN YAT-SEN UNIVERSITY, Guangzhou, China; [3]Dept. of Pathophysiology, [2]Sun Yat-Sen Univ., Guangzhou, China; [4]Zhongshan Sch. of Med., Sun Yat-Sen Univ., Guangzhou City, China

Abstract: Transient sensory experiences can evoke sustained emotional disturbances, yet the neural mechanisms driving this transition remain poorly understood. Here, we demonstrate that acute pain triggers persistent anxiety extending beyond nociceptive input, mediated by diminished oxytocin signaling in the dorsal bed nucleus of the stria terminalis (dBNST). Restoring oxytocin receptor activation in the dBNST robustly alleviated anxiety-like behaviors in mice after acute pain subsided. Mechanistic investigations revealed that somatostatin-expressing (SST) neurons in the dBNST ($dBNST^{SST}$ neurons) are essential for oxytocin's anxiolytic effects, as chemogenetic inhibition of $dBNST^{SST}$ neurons abolished benefits of oxytocin. Furthermore, transcriptomic analyses identified oxytocin-dependent regulation of genes involved in synaptic transmission and neuronal excitability, suggesting a mechanism by which oxytocin counteracts maladaptive plasticity. Collectively, our findings outline a multi-level framework—encompassing molecular, cellular, and circuit mechanisms—that explains how acute sensory stimuli drive long-term emotional dysregulation. This work advances our understanding of pain-related affective disorders and highlight oxytocin signaling and $dBNST^{SST}$ neurons as promising therapeutic targets.

Disclosures: Y. Xiaodan: None. **S. Fang:** None. **L. Huang:** None. **B. Li:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Title: The effects of systemic oxytocin injection on emotional contagion of chronic pain in male and female mice

Authors: I. S. DIAS[1], F. PEREIRA MONTEIRO[2], ***A. CANTO-DE-SOUZA**[3,2,4];
[1]Dept. de Psicologia - DPsi, Univ. Federal de São Carlos - UFSCar, São Carlos, Brazil; [2]2Joint Grad. Program in Physiological Sci. UFSCar/UNESP, São Carlos, Brazil; [3]Psychobiology Group, Dept of Psychology, UFSCar, São Carlos, Brazil; [4]3Program in Psychology UFSCar, São Carlos, São Paulo, São Carlos, Brazil

Abstract: Our research group has shown that male observer mice that live in pairs with a conspecific, subjected to chronic pain, show increased anxiogenic-like response, assessed in the elevated plus-maze (EPM), and nociception, indicating that anxiety and pain can be socially modulated. In addition, research has shown that oxytocinergic neurotransmission can modulate social and affective behaviors, cognition, and responses to pain. We investigated the effects of systemic oxytocin (OT) injection on the emotional contagion of pain in male and female mice that cohabited with a conspecific on chronic pain. Male and female Swiss mice (n=6-9/group) were housed in quartets for 28 days. On day 14, one mouse per quartet was assigned to either cagemate nerve constriction (CNC) by sciatic nerve constriction (SNC) or cagemate sham (CS) by sham surgery. The remaining three cagemates became observer CNC (Ob-CNC) or observer sham (Ob-CS), respectively. From days 26-28, a barrier was introduced into the cages for 15 minutes daily. On day 28, Ob-CNC and Ob-CS mice received a subcutaneous (s.c.) injection of saline or OCT (0.5, 1.0, or 2.0 mg/kg). Thirty minutes later, they subjected the EPM test for 5 minutes. Immediately after the EPM, they received an intraperitoneal (i.p.) injection of 0.6% acetic acid for a 5 minutes writhing test. The hot plate test was used to confirm the effectiveness of the constriction surgery in the CNC group. The hot plate test revealed that the SNC procedure increased pain response in male and female CNC cagemates. The three doses of OT decreased the number of abdominal contortions in CNC observer mice for males and females, compared to the Ob-CS. The EPM results demonstrated that male Ob-CNC treated with the three doses of OT increased the % open arm entries, while only OT 2.0 increased the % time spent in the OA. None of the OT doses altered the anxiety-like responses in female Ob-CNC. These results demonstrate that living in quartets with a mouse subjected to CNC induces hyperalgesia in male and female Ob-CNC. The three doses of OT attenuated the pain hypersensitivity in male and female animals that cohabited in quartets with mice subjected to chronic constriction injury. However, these

doses of OT reduced anxiety only in male Ob-CNC. This study suggests the involvement of oxytocinergic neurotransmission in modulating the emotional and pain responses in male and female mice.

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Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Program #/Poster #: PSTR406.12/HH15

Topic: H.04. Emotion

Support: Korea government grant RS-2025-00517214
Korea Government grant NRF-2021R1A6A1A03045425

Title: Adaptive balancing of approach and avoidance is regulated by the central nucleus of the amygdala

Authors: *J.-S. CHOI[1], J. LEE[2];
[1]Korea Univ., Sungbuk-Ku, Seoul, Korea, Republic of; [2]Dept. of Psychology, Univ. of California San Diego, San Diego, CA

Abstract: Foraging in the wild requires adaptive balancing between approach and avoidance. While the central nucleus of the amygdala (CeA) is implicated in processing both aversive and appetitive stimuli, its role in suppressing ongoing reward-seeking behavior in response to anticipated threats remains underexplored. Here, we used employed a dynamic foraging task in which rats had to consume a sucrose solution from a port “guarded” by a robot with a fast-acting threat motion. The mechanical attack was triggered 6 seconds after the first lick. Fiber photometry revealed that CeA activity increased during reward consumption and was further elevated in trials where animals successfully withdrew before the robot's attack. A machine-learning analysis also revealed that the neural activity predicted the success or failure to avoid the attack on a trial-by-trial basis. To test whether this heightened CeA activity was necessary for behavioral suppression, we transiently inhibited CeA activity at specific time points. Inhibiting the CeA during periods of elevated activity resulted in less avoidance: rats showed increased licking and failed to withdraw in time, despite increased vulnerability to attacks. In contrast, inhibition at other time points had no effect. These findings suggest that the CeA plays a critical role in moment-to-moment arbitration between reward pursuit and threat avoidance, shaping adaptive decisions that promote survival.

Disclosures: J. Choi: None. **J. Lee:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Program #/Poster #: PSTR406.13/II1

Topic: H.04. Emotion

Title: Neuromodulation in the interoceptive insular cortex maintains fear balance

Authors: *A. D. ZYCH, M. MALEZIEUX, E. CHO, R.-E. HUETTL, A. RESSLE, B. SCHMID, N. GOGOLLA;
Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: Maintaining emotions in an adaptive range is important for survival. Our group has previously shown, that the posterior insular cortex (pInsCtx) plays a crucial role in keeping fear in balance upon extinction learning. Surprisingly, we found that the pInsCtx exerts both, a fear extinction impeding, but also facilitating role depending on the strength of the acquired fear levels. However, we lack mechanistic understanding which factors may enable the dual role of the pInsCtx on fear extinction learning. The neuromodulators acetylcholine (ACh) and noradrenaline (NA), are known to be released in response to sensory stimuli, as well as upon changes in affective states. Furthermore, these neuromodulators have been implicated in fear and extinction learning, as well as the regulation of bodily functions. Given these roles, we here aimed at investigating a potential contribution of these neuromodulators to the role of the pInsCtx in keeping fear in balance. To understand the potential recruitment of ACh and NA within the pInsCtx during fear learning and extinction, we characterized their release using GRAB sensors during classical auditory fear conditioning in freely moving mice, as well as in head-fixed animals, where we obtained physiological readouts of heart rate, pupil and other arousal signals. Our results reveal specific differences in the dynamics of NA and ACh release in response to different fear-related sensory stimuli, changes in autonomic readouts and emotional behaviors. Strikingly, interference with neuromodulatory release in the pInsCtx during fear extinction via optogenetic terminal inhibition resulted in bidirectional effects. While inhibition of NA terminals impaired fear extinction, inhibition of ACh terminals facilitated fear extinction. Importantly, these effects were contingent upon the fear state of an animal. Together, our findings suggest that NA and ACh in the pInsCtx are crucial in regulating the adaptive balance of fear maintenance and extinction learning. This equilibrium is facilitated through the cholinergic integration of exteroceptive and interoceptive signals, alongside the noradrenergic encoding of fear state.

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Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR406.14/II2

Topic: H.04. Emotion

Title: Role of the posterior insular cortex in cardiorespiratory interoception during fear

Authors: *C. WEIAND[1], A. RESSLE[4], B. SCHMID[5], V. SPOORMAKER[2], N. GOGOLLA[3]; [1]Max Planck Inst. of Psychiatry, Munich, Germany; [2]Max Planck Inst. of Psychiatry, Muenchen, Germany; [3]Emotion Res., Max Planck Inst. of Psychiatry, Munich, Germany; [4]Max-Planck Inst. für Psychiatrie, Munich, Germany; [5]Max Planck of Psychiatry, Muenchen, Germany

Abstract: Interoception encompasses the sensing, interpretation, integration and regulation of internal signals of an individual (Chen et al, 2021). Bodily signals first reach the brainstem, especially the nucleus of the solitary tract (NTS), which is the main relay site for interoceptive signals (Gallager & Pert, 1978). Bodily signals are further transmitted to higher order subcortical and cortical brain regions (Cechetto, 2014). Most notably, the posterior insular cortex (pInsCtx), which is a viscerotopically organized cortical region (Cechetto and Saper, 1987). Interestingly, the insula is not only a visceral afferent but also a motor efferent cortex (Oppenheimer and Cechetto, 1990; Yasui et al, 1991) and receives internal and external signals (Craig, 2002, 2003; Critchley et al., 2004), which enables it to be heavily involved in interoceptive processes. Disruption of interoceptive processes, however, are associated with impairments of mental health (Critchley and Garfinkel, 2017; Garfinkel et al., 2016). For example, both, cardiac and respiratory interoception have been shown to be altered with fear and anxiety (Garfinkel et al., 2016). However, the mechanistic underpinnings of how brain-body interactions are influencing emotions is poorly understood. Here, we focused on the characterization of the interaction between cardiac and respiratory signals with the neuronal activity within the pInsCtx during conditioned and innate fear. We assessed bodily signals in freely moving mice, utilizing wireless telemetry to record ECG signals and intrapleural pressure, as a readout of breathing. Fiber photometry measurements of bulk calcium signals from the pInsCtx were used to assess neuronal activity. Results reveal that brain-body interactions are cue- and behaviour dependent. Furthermore, directionality analyses of all physiological signals with pInsCtx activity suggest a fear state dependent brain-body interaction. Interestingly, internal fear-states further influenced which pInsCtx hemisphere was more connected with the body. Additionally, brain-body interactions differ between the beginning of the fear behavior 'freezing' compared to the end of the behaviour. Overall, the data suggests that bidirectional interactions between bodily signals and pInsCtx activity represent internal fear states.

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Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Topic: I.07. Long-Term Memory

Support: NIH Grant R01MH124992

Title: Training strength modulates the temporal window of anisomycin induced disruption of contextual fear memory consolidation and reconsolidation

Authors: *J. D. JARACZEWSKI[1], H. WANG[2];
[1]Michigan State Univ., Washington, MI; [2]Michigan State Univ., East lansing, MI

Abstract: Background. De novo protein synthesis is widely believed to be necessary for memory consolidation and reconsolidation, yet much of the evidence rests upon injection of protein-synthesis inhibitors (PSIs) such as anisomycin, which have many off-target effects. Recent calcium imaging and RNA quantification data from our lab indicates anisomycin increases neural activity, a process known to cause amnesia. **Objective.** To disentangle protein synthesis inhibition from its ancillary effects, we mapped the time course over which anisomycin disrupts contextual fear memories formed by either weak or strong training, during either consolidation or reconsolidation. **Methods.** A contextual fear conditioning protocol was used in which male 10-14 week old C57BL/6 mice received weak or strong context-shock pairings. For consolidation, animals were injected i.p. with saline (1 h) or anisomycin (150 mg/kg) at 0, 1, 3, 4, or 6 h post-conditioning. For reconsolidation, the same time points (except for 4 h and 6 h) followed a 3-min retrieval 24 h after training. Freezing, measured 24 hours after training and retrieval respectively, served as a measure of memory and was scored automatically using FreezeFrame software (ActiMetrics) to eliminate experimenter bias. All data were analyzed by one-way ANOVA (n = 7-10 mice per group); Dunnett's post hoc tests were performed when the omnibus F reached significance. **Results.** *Consolidation*: Weakly trained memories were disrupted when anisomycin was given up to 4 h ($p= .019$) but not 6 h ($p= .990$) post-conditioning. Strongly trained fear memories were interfered with when anisomycin was administered immediately ($p<.001$) but not 1 h ($p= .853$) after conditioning. *Reconsolidation*: For weak training, anisomycin impaired memory at 1 h ($p = .014$) but not 3 h ($p = .907$) post-retrieval; strong memories were insensitive at all time points ($p > .05$). **Conclusions.** Stronger conditioning shortens both consolidation and reconsolidation; reconsolidation is shorter than consolidation. Results align with previous time course data for chemogenetic (Gq-DREADDS) excitation induced disruption providing evidence for an excitation based mechanism of disruption for PSIs. Future work will co-infuse anisomycin with chemogenetic silencing to determine if increased neuronal activity is required for PSI induced disruption. Clarifying this foundational question is essential for guiding translational strategies that aim to modify maladaptive memories in disorders such as PTSD.

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Poster

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Topic: I.07. Long-Term Memory

Support: GPR 2030

Title: Looking for the engram underlying PTSD-like memory: focus on the role of the anterior cingulate cortex and NMDA receptors in traumatic memory maintenance.

Authors: *F. V. SIMÕES[1], A. DESMEDT[2], L. GROC[3], O. NICOLE[4], S. TRONEL[5];
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Abstract: Recent progress has been made in finding the engram, classically represented as a population of neurons activated during memory encoding and reactivated during memory retrieval. So far, the engram that supports normal fear memory has been well studied, but the one that supports pathological memories remains elusive. Once recalled, memories enter a period of malleability, called destabilization-reconsolidation, during which they can be either reinforced or updated to change adaptively. Targeting the reconsolidation process could be a potential treatment for alleviating memories associated with conditions such as post-traumatic stress disorder (PTSD).A cardinal symptom of post-traumatic stress disorder (PTSD) is a paradoxical and long-lasting alteration in memory, including both hypermnesia for a salient but irrelevant trauma-related cue and contextual amnesia for the context in which the trauma occurred. Unlike “normal” adaptive fear memory, this “abnormal” maladaptive traumatic memory appears to be persistent and resistant to modification. This strongly suggests that the engram supporting PTSD-related memory is different from that of normal fear memory. Using a behavioral paradigm in mice that mimics the paradoxical memory impairment of PTSD, TRAP2 mice and whole brain mapping, we sought to identify the network and engram that support both maladaptive and adaptive memories. First, we found a decorrelation of the functional connectome in the formation of PTSD-like memory compared to normal fear memory. Additionally, the anterior cingulate cortex (ACC) was shown to be hyperactivated after trauma encoding. During retrieval, the ACC and basolateral amygdala (BLA) showed increased reactivation, while the dentate gyrus (DG) showed decreased reactivation. Focusing on the ACC in traumatic memory retrieval, we chemogenetically silenced the ACC engram cells before retrieval. We showed that this silencing completely disrupts the hypermnesia for trauma-related cue.We additionally show that this traumatic memory is resistant to extinction and unable to reconsolidate, probably due to an inability to destabilize. Since NMDARs are identified as key players in memory lability upon reactivation, we hypothesized that GluN2B-containing NMDARs are required to initiate the plasticity process destabilizing normal fear memory. A traumatic event could redistribute synaptic GluN2A and GluN2B NMDAR leading to further synaptic adaptations. Here, we showed that reduced GluN2A activity in ACC of PTSD-like mice before reactivation could promote memory malleability and allow reconsolidation of traumatic memory.

Disclosures: F.V. Simões: None. **A. Desmedt:** None. **L. Groc:** None. **O. Nicole:** None. **S. Tronel:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.17/II5

Topic: H.04. Emotion

Support: General Research Fund, Ref: 14119024

Title: Glp-1r agonist semaglutide alleviates neuropathic pain-induced depression via inhibition of dorsolateral septum hyperactivity

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Abstract: The glucagon-like peptide-1 receptor (GLP-1R) agonist semaglutide has gained increasing attention in mental health research due to its real-world efficacy in alleviating depressive symptoms. In this study, we demonstrate that both prophylactic and therapeutic administration of semaglutide exerts antidepressant effects in male and female mouse models of neuropathic pain-induced depression. Functional ultrasound imaging (fUSI), calcium photometry, and c-Fos staining revealed hyperactivity in the dorsolateral septum (LSD), which has been implicated in depression-like behaviors. Semaglutide treatment effectively reduced this hyperactivity. We further identified that GLP-1R is highly expressed in the LSD. Microelectrode array (MEA) recordings showed that bath application of semaglutide significantly suppressed the spiking activity of GLP-1R-positive neurons in this region. These findings suggest that semaglutide alleviates depression-like behaviors by dampening LSD hyperactivity, providing a mechanistic explanation for its antidepressant effects. Ongoing work is exploring the downstream targets of LSD using fUSI-based functional connectivity and optogenetic approaches in GLP-1R-Cre and fosTRAP mice.

Disclosures: X. Wang: None. **X. Liu:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.18/II6

Topic: H.04. Emotion

Support: 2P30DA048736-06
2T32DA007278-31

Title: Imaging the neural encoding dynamics of chronic pain transition in the ventrolateral periaqueductal gray

Authors: *M. CRITZ[1], K. ELLIOFF[1], S. C. PIANTADOSI[2], B. B. LAND[1], M. R. BRUCHAS[2,1];
[1]Pharmacol., [2]Anesthesiol. and Pain Med., Univ. of Washington, Seattle, WA

Abstract: Chronic pain affects up to 100 million people in the United States, and severely impacts quality of life. Current analgesic treatments such as opioid drugs have significant drawbacks, making the management of chronic pain uniquely difficult. There is a significant need for new chronic pain treatments, as well as better understanding of the neurobiological and pharmacological mechanisms governing the development of chronic pain. The ventrolateral periaqueductal gray (vlPAG) is an important hub for the descending modulation of pain signals, with both excitatory (vGlut2) and inhibitory (vGAT) neurons regulating "on and off" pain responses. Prior research efforts have not been able to track individual neurons in abundance across the PAG, during transition to chronic pain. Therefore, here we used in vivo 2-photon resonance microscopy, coupled to microprism technology to observe large scale calcium dynamics of thousands of vlPAG neurons in real time during the development of chronic pain in a partial sciatic nerve ligation model. Concurrently, we used a head-fixed running wheel, with time-locked nociceptive stimuli to characterize pain and escape behaviors across chronic pain development. Additionally, the vlPAG expresses an abundance of inhibitory GPCRs, including cannabinoid receptors (CB1), and may therefore be an important target for the now common use of phytocannabinoids (i.e. THC) to relieve chronic pain. We also examined here whether phytocannabinoid treatment influences these patterns. We also investigated the impact of acute THC treatment on the activity of these neurons and the manifestation of pain responses. These studies inform how the vlPAG encodes pain to confer analgesia, how these neurons transition in pain states, and examine how the endocannabinoid system modulates these interactions.

Disclosures: M. Critz: None. **K. Ellioff:** None. **S.C. Piantadosi:** None. **B.B. Land:** None. **M.R. Bruchas:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR406.19/II7

Topic: H.04. Emotion

Support: NIH Grant R01DK132566

Title: Leptin-responsive ventral tegmental area circuit mediates inflammatory pain

Authors: J. JUNG, *S. SHIN;
Virginia Tech., Roanoke, VA

Abstract: Chronic inflammation can severely impair quality of life by driving persistent pain in patients with inflammatory diseases. While central mechanisms such as altered synaptic plasticity and neuronal responsiveness contribute to inflammatory pain, how specific neural pathways respond to systemic inflammation to exert top-down control over pain remains poorly understood. Here, we demonstrate in mice that downregulation of the leptin receptor (Lepr) in the ventral tegmental area (VTA) selectively attenuates inflammatory pain responses. Lepr-expressing VTA (Lepr VTA) neurons exhibit increased activity during the inflammatory pain phase. We further identify two distinct Lepr VTA neuronal circuit projecting to either the lateral habenula (LHb) or bed nucleus of the stria terminalis (BNST). Notably, selective inhibition of Lepr VTA neurons projecting to the LHb suppresses inflammatory pain responses. These findings reveal a leptin-responsive pathway that mediates inflammation-induced pain sensitization and highlight a potential circuit-based target for pain modulation.

Disclosures: J. Jung: None. **S. Shin:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR406.20/II8

Topic: H.04. Emotion

Title: Wheel access alters anxiety- and fear- related behavior in C57BL/6J and DBA2/J mice.

Authors: *C. HARTLESS[1], G. D. BLAHUSIAK[2], M. N. COOK[3];
[1]Univ. of Memphis, Memphis, TN; [2]Tulane Univ., New Orleans, LA; [3]Psychology, Univ. Memphis, Memphis, TN

Abstract: Physical activity has been associated with positive mental health outcomes. In many instances, exercise is used therapeutically to counteract the effects of stressful experiences however, less is known about the potential protective effects of exercise. While some animal models have shown that voluntary wheel running (VWR) can decrease anxiety and fear related behaviors, few have explored how genetic background, and sex can influence these effects. The goal of the current project is to characterize how 1 week of VWR can influence anxiety- and fear-related behaviors in male and female C57BL/6J (B6) and DBA2/J (D2) mice. Mice were single housed for 7 days in either standard cages or in cages with a running wheel. On day 8, mice were tested in the Elevated Zero Maze (a.m.) and Open Field (p.m.). On day 9, mice underwent fear conditioning and then contextual fear on day 10. There were no strain or sex differences in distance or speed when running. D2 mice with access to VWR entered an open quadrant of the Elevated Zero Maze quicker and habituated to the Open Field, when compared to

controls. VWR did not affect behavior in the Elevated Zero Maze or Open Field in B6 mice. VWR attenuated the contextual fear response in B6 and D2 mice. Sex did not influence the behaviors measured after the VWR paradigm. The effects of VWR on anxiety-related behavior were strain dependent while VWR produced similar effects on fear-related behaviors in both strains. These findings suggest the need to expand upon how different durations of VWR can affect behavior and how sex and genetics can influence the expression of anxiety- and fear-related behaviors.

Disclosures: C. Hartless: None. **G.D. Blahusiak:** None. **M.N. Cook:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.21/II9

Topic: H.04. Emotion

Support: NSERC

Title: Predator Scent, Prey Response: The Effects of Different Concentrations of Trimethylthiazoline on Behaviour and Neural Activity.

Authors: *Z. SIDDIQI, D. C. BOORMAN, L. J. MARTIN;
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Abstract: Trimethylthiazoline (TMT) is a single molecule component of fox feces that induces innate defensive behaviours such as freezing in rodents, making it a widely used environmental stress model in laboratory research. However, current literature exhibits a lack of consistency in the TMT concentrations that have been used across studies, if they are reported at all, leading to variability in experimental conditions and the results observed. Additionally, the absence of a well-defined dose-response curve complicates the identification of an optimal dose of TMT for eliciting behavioural responses. Here, we characterized a set of defensive behaviours commonly observed in mice during TMT exposure using 3%, 10%, and 30% TMT, with similar concentrations of butyric acid (BA) and reverse osmosis (RO) water as controls. Our findings demonstrate that 10% TMT elicited the most robust freezing response, followed by 30% TMT, indicating a non-linear relationship between concentration and defensive behaviour. These results suggest that higher concentrations of TMT proportionally increase the number of defensive behaviours evoked. Additionally, preliminary findings suggest differences in neural activity across groups as measured by the expression of the IEG c-Fos. Therefore, standardizing TMT concentrations is crucial for ensuring consistency and reproducibility in future research.

Disclosures: Z. Siddiqi: None. **D.C. Boorman:** None. **L.J. Martin:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR406.22/II10

Topic: G.03. Stress and the Brain

Support: UNAM PAPITAI202427

Title: Innate behavioral responses in the autism mouse model C58J to inescapable predator odor exposure

Authors: *E. CALLADO[1], E. LUNA CASTAÑÓN[2], E. ROCHA GONZÁLEZ[3], O. R. HERNANDEZ PEREZ[4];
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Abstract: The ability to detect and respond to predator odors is fundamental for mammalian survival. Innate defensive responses—including avoidance behaviors, hyperactivity, and physiological changes—are mediated by specific sensory and neurobiological systems. Interestingly, these responses are affected in individuals with autism spectrum disorder (ASD). In this context, C58J mice have been employed as a natural model for autism. In this study, we investigated how C58J mice react to exposure to predator-associated odors, specifically cat urine odor. Using a controlled experimental design, we exposed C58J mice to the scent of cat urine and measured both behavioral responses (such as avoidance and exploratory activity). Our results demonstrate that C58J mice exhibit clearly differentiated innate defensive responses to predator odors, characterized by short latency times, increased risk assessment behaviors, behavioral freezing, and repetitive behaviors. These findings suggest that C58J mice process threatening stimuli differently compared to typical mice, which may reflect alterations in their threat perception and response mechanisms. Overall, this research provides insight into the neurobehavioral processing of danger cues in a model relevant to ASD, with potential implications for understanding the neural basis of threat response alterations in autism.

Disclosures: E. Callado: None. **E. Luna Castañón:** None. **E. Rocha González:** None. **O.R. Hernandez Perez:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR406.23/II11

Topic: H.04. Emotion

Support: PhD Fellowship from the Argentine National Research Council (CONICET)
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FONCYT – Agencia I+D+I (PICT 2018-0835, 2018-0003)
CONICET (PIP 2020-0923)

Title: Very Important Players: Dissecting a dopamine-sensitive microcircuit involving VIP interneurons in pain modulation within the anterior insular cortex

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Abstract: Pain is an emotional and subjective experience that arises from distributed brain activity. The Anterior Insular Cortex (AIC) acts as a central anatomical hub for the integration of sensory, emotional, motivational and cognitive functions, including pain perception. The mesolimbic dopaminergic system is known for its role in processing motivationally relevant events and modulating pain perception. In particular, dopamine decreases nociceptive sensitivity through D1 receptor (D1R) signaling in the AIC (Burkey et al., 1999). However, the cellular and microcircuit mechanisms through which dopamine modulates AIC activity during pain remain largely unknown. To address how dopamine influences AIC microcircuits during pain, we designed two complementary objectives combining anatomical, functional and behavioral approaches. Using D1R-Tomato transgenic mice, we found that D1 receptor-expressing (D1R+) neurons in the superficial layers of the AIC are predominantly inhibitory interneurons, whereas those in deeper layers include both pyramidal cells and interneurons. Combining ex vivo electrophysiology with morphological reconstructions, we demonstrated that D1R+ interneurons exhibit bipolar dendritic morphology, irregular firing patterns and are excited by D1R agonists. These features are characteristic of a specific subclass of interneurons expressing vasoactive intestinal peptide (VIP). Supporting this, immunohistochemistry revealed that the majority of D1R+ neurons in superficial layers were VIP+, with none co-expressing parvalbumin or somatostatin, markers of other major interneuron populations. Given the role of VIP+ interneurons in disinhibitory circuits by inhibiting other interneurons, dopaminergic modulation via D1Rs likely influences AIC microcircuit dynamics broadly. Dopamine may enhance AIC output by directly exciting D1R+ pyramidal neurons and indirectly through VIP+ interneuron-mediated disinhibition. In parallel, to test functional relevance, we combined intra-AIC microinjections of a D1R-selective agent with behavioral assays. Mice underwent open field testing after acute abdominal pain induction via 1% acetic acid injection. We quantified distance traveled, immobility, and abdominal constrictions as nociceptive and affective pain markers, hypothesizing that D1R activation would reduce pain-related immobility and constrictions. These findings provide new insights into dopamine's modulation of AIC microcircuits during pain, advancing understanding of how D1R pathways integrate affective and sensory components.

Disclosures: M. Trujillo: None. **F. Kasanetz:** None.

Poster

PSTR406: Fear, Anxiety, and Pain in Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR406.24/II12

Topic: H.04. Emotion

Support: NIH Grant R01MH123523

Title: Single-cell insights into gene regulation in the mouse brain across the estrous cycle and sex

Authors: M. C. TICKERHOOF[1], L. DEMARCHI[1], A. SISTA KAMESHWAR[2], L. OULDIBBAT[1], M. SUZUKI[2], ***M. KUNDAKOVIC**[1];
[1]Biol. Sci., Fordham Univ., Bronx, NY; [2]Dept. of Nutr., Texas A&M Univ., College Station, TX

Abstract: Anxiety and depression disorders are two times more prevalent in women than in men. While it is known that ovarian hormone shifts play an important role in this sex bias, the underlying molecular mechanisms remain underexplored. To this end, we previously showed that anxiety-related behaviors vary across the estrous cycle in mice, and we linked these changes to changes in chromatin and gene expression in the ventral hippocampus (vHIP), the region critical for emotion regulation in rodents. In the current study, we characterized gene expression and chromatin accessibility in the mouse vHIP at single-nucleus resolution across sex and the estrous cycle, focusing on diestrus (low estradiol-high progesterone) and proestrus (high estradiol-low progesterone). Nuclei from 11-week-old diestrus and proestrus females and males (N=6 mice or 3 biological replicates/group) were processed using the 10 X Chromium Single-Cell Multiome kit. Using Seurat, we identified 60 cellular clusters in the vHIP, including subclusters of excitatory and inhibitory neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells. We revealed sex- and estrous cycle-dependent changes in cellular proportions among these clusters, including hormone status-driven cellular changes in the dentate gyrus. While the highest number of cycle-dependent differentially expressed genes (DEGs) were found in excitatory neurons, we found extensive chromatin changes across the cell types. Within specific cell types, we identified differential gene expression and chromatin accessibility between the sexes and across the cycle, including genes relevant for synaptic function, neurogenesis, memory, and behavior. We identified the gene encoding transthyretin (*Ttr*), a thyroid hormone transporter, as an important sex- and hormone state-dependent candidate gene. We then overexpressed *Ttr* in excitatory vHIP neurons of female mice to show its functional relevance for behavioral changes across the estrous cycle. These results provide single-cell resolution insights into sex- and estrous cycle-dependent gene regulation relevant to emotion regulation and neuroplasticity. This work informs the neurobiology underlying sex bias in depression and anxiety disorders, paving the way for precision medicine approaches taking hormonal state into account.

Disclosures: M.C. Tickerhoof: None. **L. Demarchi:** None. **A. Sista Kameshwar:** None. **L. Ouldibbat:** None. **M. Suzuki:** None. **M. Kundakovic:** None.

Poster

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Program #/Poster #: PSTR406.25/II13

Topic: H.03. Motivation

Support: The present work was supported by the Intramural Research Program of NIDA, NIH

Title: An open-source, budget-friendly tail suspension test platform with high temporal resolution for behavioral and neural correlation studies

Authors: *Z. YE, X. MIN, S. IKEMOTO;
Intramural Res. Program of NIDA, NIH, Baltimore, MD

Abstract: The tail suspension test (TST) is a widely used assay for assessing stress-coping behaviors in rodents by subjecting mice to an acute, inescapable stressor. During the test, animals exhibit alternating active (struggling) and passive (immobile) behaviors, which serve as reliable proxies for screening antidepressant compounds. Here, we present an open-source, low-cost, and scalable platform for automated quantification of active and passive coping behaviors during the TST. The apparatus features four independent compartments, allowing simultaneous testing of up to four mice. A central stage facilitates secure tail attachment and accommodates head-mounted equipment, such as fiber photometry or optogenetic cables, ensuring compatibility with neural recording and manipulation techniques. Each suspension bar is equipped with a load cell sensor, interfaced with an Arduino Uno and controlled via a Bonsai-Rx software suite. The system records behavioral signals at 80 Hz, providing high temporal resolution for alignment with neural data. A custom Python-based algorithm processes the load cell outputs to extract periods of movement and immobility. To validate the system, we performed a double-blind comparison between sensor-derived measurements and manual video-based scoring. The load cell signals showed a strong correlation with human annotations (r^2=0.9978, $p<0.0001$). As a proof of concept, we tested the effects of the antidepressant imipramine (15 mg/kg, i.p.), which significantly reduced immobility compared to saline controls ($p<0.0001$, n=8 for each group). In summary, our automated TST platform enables efficient, high-throughput behavioral assessment with minimal experimenter bias. All hardware schematics, firmware, and analysis code will be freely available to the neuroscience community.

Disclosures: Z. Ye: None. **X. Min:** None. **S. Ikemoto:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.01/II14

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Elucidating transcript dynamics in post-traumatic stress disorder in human brains

Authors: *J. SOTO-VARGAS[1], J. WANG[2], Y. LIU[2], X. SHI[2], M. SKARICA[3], M. GIRGENTI[4];
[1]INP, Yale Univ., New Haven, CT; [2]Psychiatry, Yale Sch. of Med., New Haven, CT; [3]Neurosci., [4]Psychiatry, Yale Med. Sch., New Haven, CT

Abstract: Post-traumatic stress disorder (PTSD) is a trauma-based disorder with a prevalence of 7.5% among civilian populations. It co-occurs with major depressive disorder in >50% of diagnosed patients. Although they are distinct stress disorders, PTSD and major depressive disorder (MDD) share overlapping symptoms, etiology, and gene expression signatures. The role of alternative splicing of transcripts in PTSD within the fear circuitry of the brain remains poorly understood. We performed RNA sequencing in four brain regions: dorsal prefrontal cortex (DLPFC), dorsal anterior cingulate cortex (dACC), basolateral amygdala (BLA), medial amygdala (MeA) in postmortem human brain across three diagnoses, including neurotypical controls (n = 109/region), MDD (n = 109/region) and PTSD (n = 107/region). We conducted differential gene expression (DGE), transcript/isoform expression (DTE), and transcript/isoform usage (DTU), as well as differential intron usage (DIU) analyses. Our preliminary findings revealed a high correlation of differential gene expression between PTSD and MDD (R^2 = 0.50-0.65), while analysis at the transcript/isoform-level was lower (R^2 = 0.30-0.37), capturing putative isoform-specific regulation between disorders. Across all regions, PTSD showed a higher number of unique differentially expressed transcripts (DETs) compared to MDD, with limited overlap between disorders, especially in MeA, where only 12.1% of downregulated and 7.7% of upregulated DETs were shared. DIU analysis revealed weak negative correlation of signatures for case-control comparisons at the splicing-level between PTSD and MDD (R^2 = 0.13-0.18). Distinct splicing profiles are further supported by the low overlap (3-6% across regions) in significant splicing events. We observed divergent splicing between disorders for 1853 genes, including *NRXN2* in MeA. *NRXN2* shows a mutually exclusive splicing event between exon 17 and exon 18 that is differentially spliced for both MDD (FDR = 0.04) and PTSD (FDR = 0.02). In PTSD, exon 18 is included (ΔPSI = +0.06) while exon 17 is skipped (ΔPSI = -0.06), with the opposite observed in MDD, where exon 17 is included (ΔPSI = +0.04). This converse splicing regulation in *NRXN2* underscores a potential isoform-level mechanism that distinguishes PTSD from MDD within fear-related circuitry in MeA. These findings suggest that PTSD and MDD exhibit distinct transcriptomic regulation profiles across fear-related brain regions, with minimal overlap in transcript expression and divergent splicing regulation not captured at the gene level.

Disclosures: J. Soto-Vargas: None. **J. Wang:** None. **Y. Liu:** None. **X. Shi:** None. **M. Skarica:** None. **M. Girgenti:** None.

Poster

PSTR407: Human Studies: Trauma

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Program #/Poster #: PSTR407.02/JJ1

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Microglia morphometric analyses reveal phenotypic differences between PTSD and MDD in human brain

Authors: *N. F. DENNIS[1], S. LIU[2], M. J. GIRGENTI[3];
[1]Interdepartmental Neurosci. Program, [2]Neurosci., Yale Univ., New Haven, CT; [3]Psychiatry, Yale Med. Sch., New Haven, CT

Abstract: Post-traumatic stress Disorder (PTSD) is a complex disorder occurring in the aftermath of severe trauma. PTSD has been linked to altered susceptibility to other diseases and can have long-term effects on biological processes, including the inflammatory response. Neuroinflammatory factors and cytokines are dysregulated in people with trauma disorders. Microglia, the resident immune cells of the brain, have altered cytokine production and function in chronic stress. Using postmortem tissue collected from the dlPFC (dorsolateral prefrontal cortex) of donors with PTSD and major depressive disorder (MDD), we stained for IBA1, a marker of microglia, and UBA1, an antibody for vasculature. Preliminary findings show an increased number of microglia in MDD and a decrease in PTSD compared to non-psychiatric controls. At higher magnifications, we discern morphological properties (area, perimeter, circularity) to demonstrate the activation state (ramified, reactive, primed, amoeboid) of microglia and determine if there are distinct phenotypic differences between disease states. Morphologically, the cell body size is larger in those with MDD compared to PTSD, suggesting differences in the microglia activation state. Additionally, we performed sex-specific analyses. We found females have smaller microglia cell body areas in PTSD compared to MDD and compared to males of the same diagnostic groups. These findings suggest microglial dynamics are different in PTSD and MDD brains, which may be sex-specific.

Disclosures: N.F. Dennis: None. **S. Liu:** None. **M.J. Girgenti:** None.

Poster

PSTR407: Human Studies: Trauma

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Program #/Poster #: PSTR407.03/JJ2

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Multiomic Single-cell dissection reveals critical regulators in the human PTSD and MDD brain

Authors: *X. SHI[1], J. WANG[2], J. SOTO-VARGAS[1], M. GIRGENTI[3];
[1]Yale Univ., New Haven, CT; [2]Psychiatry, Yale Sch. of Med., New Haven, CT; [3]Psychiatry, Yale Med. Sch., New Haven, CT

Abstract: Post-traumatic stress disorder (PTSD) and major depressive disorders (MDD) exhibit high comorbidity, yet a detailed understanding of their associated and distinct transcriptional alterations-particularly in a cell-type-specific context, remains limited. Here we performed integrated single-nucleus RNA sequencing and chromatin accessibility profiling on >1 million nuclei from the anterior cingulate cortex (Brodmann are 24) of 111 human postmortem brains. By integrating transcriptomics, chromatin accessibility and genotype, we identified both known and novel genes associated with disease risk. Our analysis revealed disease-associated shifts in transcription in excitatory neurons, specifically in pathways involved in neuron projection development, synapse and dendrite function. We identified 1,041 differentially expressed genes associated with PTSD across all cell types and 1245 with MDD. Of these, 542 genes (31%) overlapped between the two conditions. Notably, IL7 in non-neuronal cells and PDE5A in inhibitory neurons were altered in both disorders. PTSD-specific differentially expressed genes included PYCR2 in endothelial cells and DPYD in inhibitory neurons. While MDD-specific alterations included APLP1 in inhibitory neurons and PTPRR in non-neuronal cells. Altogether, these findings provide a comprehensive mutiomic single-cell atlas of PTSD and MDD in a discrete region of the prefrontal cortex, uncovering disease-specific and shared cellular programs. These insights lay the groundwork for future precision therapeutics targeting cell-type-specific vulnerabilities in the psychiatric disorders.

Disclosures: X. Shi: None. **J. Wang:** None. **J. Soto-Vargas:** None. **M. Girgenti:** None.

Poster

PSTR407: Human Studies: Trauma

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Program #/Poster #: PSTR407.04/JJ3

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Single-nucleus multiomic atlas of the human medial amygdala reveals cell type-specific molecular alterations in ptsd brain

Authors: *J. WANG[1,2], X. SHI[2], M. SKARICA[2], J. SOTO-VARGAS[2], M. GIRGENTI[2];
[1]Yale Univ., New Haven, CT; [2]Psychiatry, Yale Sch. of Med., New Haven, CT

Abstract: Post-traumatic stress disorder (PTSD) is a prevalent and debilitating psychiatric condition, yet the molecular mechanisms by which the amygdala—specifically the medial amygdala—contribute to trauma-related pathology remain a major research gap. Medial amygdala is essential in processing threat and emotional stimuli, especially fear and stress signals, and has been reported to show transcriptomic changes in response to PTSD. Here we present the findings of a large-scale single-nucleus multiomic study profiling over 1 million

nuclei from the medial amygdala (MeA) of 108 human postmortem brains, including individuals with and without PTSD, with major depressive disorder (MDD) as a psychiatric control group. By integrating transcriptomic and epigenomic data, we defined distinct neuronal and non-neuronal cell clusters and generated a high-resolution cell type atlas with marker genes for the human medial amygdala. We further identified cell type-specific gene expression changes and chromatin assemblies associated with PTSD. Notably, we uncovered PTSD-associated differentially expressed genes in inhibitory and excitatory neurons, as well as astrocytes, implicating pathways related to GABAergic signaling and cell adhesion molecules. Differential chromatin accessibility analyses aligned with our transcriptomic findings and we were able to identify both cell type- and disease-specific cis-regulatory elements. We further identified alterations in cell-to-cell communication between excitatory and inhibitory neurons and fine mapping of PTSD alleles revealed cell type specificity of the risks. Overall, this study provides a comprehensive cell type-resolved map of molecular dysregulation in the MeA, offering new insights into the regulatory architecture of PTSD within fear-related neural circuits.

Disclosures: J. Wang: None. **X. Shi:** None. **M. Skarica:** None. **J. Soto-Vargas:** None. **M. Girgenti:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.05/JJ4

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Support: NINDS UH3NS107673

Title: Cognitive outcomes of closed-loop amygdala stimulation in treatment-resistant PTSD

Authors: *J. BAHAM[1], A. I. JANG[2], J. A. SCHNEIDERS[3], A. PERIS-YAGUE[7], G. VILCHEZ[8], R. KOEK[1], E. EINSTEIN[1], M. VALLEJO[8], J. GILL[9], M. STANGL[10], S. HILLER[1], U. TOPALOVIC[8], M. SEEBER[4], V. JANOVSKY[11], N. HASULAK[12], J. W. CHEN[5], S. E. KRAHL[1], N. A. SUTHANA[6], J.-P. LANGEVIN[1];
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Abstract: Deep Brain Stimulation (DBS) targeting the amygdala has shown promise in alleviating symptoms in patients with treatment-resistant post-traumatic stress disorder (PTSD)[1], with no worsening in any cognitive domain after four years of continuous bilateral DBS in two individuals[1,2]. However, concerns remain regarding potential cognitive side effects from chronic stimulation or neurosurgical intervention. Previous reports of verbal fluency decline in

Parkinson's patients receiving subthalamic nucleus DBS, both with stimulation on and off[3,4], underscore the need for systematic cognitive monitoring in neurostimulation trials. Responsive neurostimulation (RNS), which delivers stimulation only in response to detected neural activity (closed-loop stimulation), may offer a more targeted and cognitively safer alternative. In epilepsy patients, RNS has been associated with improvements in naming and verbal learning[5]. We report here on the efficacy and cognitive impact of RNS in three Veterans with treatment-resistant combat PTSD enrolled in our ongoing trial[2]. Symptoms were assessed using the Clinician-Administered PTSD Scale (CAPS), and comprehensive neuropsychological testing was conducted at baseline and at 6, 12, and 24 months post-implantation. Tests were grouped into domains of episodic memory, attention/working memory, and global functioning, with principal components analysis used to derive summary scores. Mixed-effects linear regression revealed a significant reduction in PTSD symptoms (mean 48% reduction in CAPS, $p = 0.017$) and improvement in global functioning ($p = 0.0043$) over time. Importantly, there were no declines in memory ($p = 0.28$) or attention ($p = 0.70$). Moreover, reductions in PTSD symptoms were associated with improvements in episodic memory ($p = 0.043$), independent of time, suggesting cognitive gains may result from symptom relief rather than direct effects of stimulation or surgery. These findings provide preliminary support for the cognitive safety of closed-loop amygdala stimulation and highlight its potential to improve memory in parallel with PTSD symptom reduction. **References:**1. Koek et al., 2024. *J Psychiatr Res*. 175:131-92. Gill et al., 2023. *Nat Commun* 14, 29973. Castelli et al., 2006. *Eur. Neurol.* 55, 136-1444. Okun et al., 2009. *Ann. Neurol.* 65, 586-5955. Loring et al., 2015. *Epilepsia*. 56, 1836-44

Disclosures: J. Baham: None. **A.I. Jang:** None. **J.A. Schneiders:** None. **A. Peris-Yague:** None. **G. Vilchez:** None. **R. Koek:** None. **E. Einstein:** None. **M. Vallejo:** None. **J. Gill:** None. **M. Stangl:** None. **S. Hiller:** None. **U. Topalovic:** None. **M. Seeber:** None. **V. Janovsky:** None. **N. Hasulak:** None. **J.W. Chen:** None. **S.E. Krahl:** None. **N.A. Suthana:** None. **J. Langevin:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.06/JJ5

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Support: UH3 NS107673
R01 MH124761-01A1

Title: Closed-loop Neuromodulation of Amygdala-Hippocampus Circuity for Treatment-Refractory PTSD: Insights from Chronic Ambulatory Monitoring in Naturalistic Settings

Authors: *M. VALLEJO[1], A. I. JANG[3], U. TOPALOVIC[1], J. GILL[10], S. HILLER[4], J. A. SCHNEIDERS[5], J. BAHAM[3], E. EINSTEIN[4], H. N. ZUBAIR[6], M. STANGL[11], C. S. INMAN[12], M. SEEBER[7], C. ORAGWAM[13], R. MUSTAPHA[4], V. R. RAO[14], N. HASULAK[15], A.

ADHIKARI[8], M. S. FANSELOW[16], M. CRASKE[4], S. E. KRAHL[4], M. VICK[17], N. CHERRY[17], J. W. CHEN[9], D. ELIASHIV[4], V. JANOVSKY[18], R. KOEK[4], N. A. SUTHANA[2], J.-P. LANGEVIN[4];
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Abstract: Treatment options remain limited for individuals with post-traumatic stress disorder (PTSD) who remain severely symptomatic despite standard treatments. Building on our prior study involving two participants[1], we investigated responsive neurostimulation (RNS) in five participants with treatment-refractory PTSD, each implanted with permanent electrodes targeting the bilateral amygdala and hippocampus. Stimulation was delivered in a closed-loop fashion, triggered by elevations in low-frequency activity (2-18 Hz) detected during naturalistic, real-world conditions, with monitoring extending over multiple years in some. In the month before stimulation onset, chronic ambulatory recordings showed that self-reported negative emotional states (e.g., flashbacks, triggers, or other negative symptoms) were consistently associated with increased bilateral amygdala power in the theta (4-8 Hz) and gamma (30-90 Hz) bands compared to neutral periods (negative vs. neutral events, $p<0.05$). Following RNS activation, participants exhibited marked reductions in amygdala theta power during both negative episodes and neutral intervals (1-month pre- vs. post-stimulation-on, $p<0.05$). We observed evolving patterns in the timing of stimulation-triggering events, with apparent shifts in daily variability. These qualitative changes appeared to track with clinical improvement; follow-up analyses will formally assess this relationship and potential progressive entrainment of dysregulated amygdala-hippocampal dynamics. Clinically, all participants had marked symptom reduction, averaging a 58% drop in CAPS-5[2] scores well above the standard 30% threshold for treatment response, with 4 of 5 meeting criteria for remission[3]. These findings provide the first evidence of long-term, closed-loop neuromodulation in PTSD and demonstrate the feasibility of real-world neurophysiological monitoring and intervention targeting the amygdala-hippocampal network. Neural activity in this circuit may serve as both a biomarker of pathological emotional states and a therapeutic target.**References:1.** Gill *et al.* A pilot study of closed-loop neuromodulation for treatment-resistant post-traumatic stress disorder. *Nat Commun* 14, 2997 (2023).**2.** Weathers et al. The Clinician-Administered PTSD Scale for DSM-5 (CAPS-5): Development and initial psychometric evaluation in military veterans. *Psychol. Assess.* 30, 383-395 (2018).**3.** Marx et al. Reliable and clinically significant change in the clinician-administered PTSD Scale for DSM-5 and PTSD Checklist for DSM-5 among male veterans. *Psychol Assess*. 34, 197-203 (2022).

Disclosures: M. Vallejo: None. **A.I. Jang:** None. **U. Topalovic:** None. **J. Gill:** None. **S. Hiller:** None. **J.A. Schneiders:** None. **J. Baham:** None. **E. Einstein:** None. **H.N. Zubair:** None. **M. Stangl:** None. **C.S. Inman:** None. **M. Seeber:** None. **C. Oragwam:** None. **R. Mustapha:** None. **V.R. Rao:** None. **N. Hasulak:** None. **A. Adhikari:** None. **M.S. Fanselow:** None. **M. Craske:** None. **S.E. Krahl:** None. **M. Vick:** None. **N. Cherry:** None. **J.W. Chen:** None. **D. Eliashiv:** None. **V. Janovsky:** None. **R. Koek:** None. **N.A. Suthana:** None. **J. Langevin:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.07/JJ6

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Posttraumatic Stress and White Matter Microstructure

Authors: *M. THOMPSON[1], A. BOLARAM[3], D. K. GREY[3], C. SANDERS[4], H. E. DARK[6], J. B. PURCELL[2], C. EICKSTEAD[1], A. KNIGHT[4], D. C. KNIGHT[5];
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Abstract: Posttraumatic Stress Disorder (PTSD) is a debilitating psychiatric disorder marked by intrusive memories, avoidance, negative alterations in mood and cognition, and hyperarousal. Treatments for PTSD are not universally effective, and a better understanding of the neurobiological processes that underlie PTSD may offer new insights that may advance future treatment approaches. The present study investigated the microstructure of white matter tracts in participants with PTSD and healthy controls using free water elimination diffusion tensor imaging. Participants (n=55, mean age=26.64) completed the Life Events Checklist and Clinician-Administered PTSD Scale for DSM-5 to assess trauma exposure and symptom severity, then were separated into PTSD (n=32) and Control (n=23) groups according to their responses. Magnetic resonance imaging data were acquired on a 3T Siemens Prisma scanner, including T1-weighted anatomical images (MPRAGE; TR/TE=2400/2.22ms, flip angle=8°, 0.8mm slices) and diffusion-weighted images (98 directions, b=3000 s/mm², TR/TE=3230/89.2ms, 1.5mm isotropic, multiband factor=4). Neuroimaging assessed tissue-specific fractional anisotropy (FAt) and tissue-specific mean diffusivity (MDt) using bi-tensor diffusion modeling and voxel-wise analyses using Tract-Based Spatial Statistics. Results revealed significantly greater FAt in the PTSD than Control group within the superior longitudinal fasciculus, cingulum bundle, and stria terminalis/fornix. Greater MDt was observed within the superior longitudinal fasciculus, cingulum bundle, uncinate fasciculus, and right stria terminalis/fornix in the Control than PTSD group. In contrast, greater MDt was observed within the left stria terminalis/fornix in the PTSD than Control group. Additionally, a negative relationship was observed between PTSD symptom severity and FAt within the superior longitudinal fasciculus, cingulum bundle, and left stria terminalis/fornix. A negative relationship was also observed between PTSD symptom severity and MDt within the left stria terminalis/fornix, while a positive relationship was observed between PTSD symptom severity and MDt within the left superior longitudinal fasciculus. In general, the greater FAt and lower MDt in the PTSD group suggest the microstructure of the white matter in these tracts may be associated with PTSD symptomology.

Disclosures: M. Thompson: None. **A. Bolaram:** None. **D.K. Grey:** None. **C. Sanders:** None. **H.E. Dark:** None. **J.B. Purcell:** None. **C. Eickstead:** None. **A. Knight:** None. **D.C. Knight:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.08/JJ7

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: The relationships among PTSD, heart rate variability, and heart rate in a cohort of women in a shelter for domestic abuse

Authors: *R. L. LLOYD[1], S. BLOSSER[2], A. MCCARTY[1];
[1]Psychology, Univ. of Minnesota, Duluth, Duluth, MN; [2]Psychology, Univ. of Minnesota, Duluth, Tavares, FL

Abstract: The present study recorded 5 minutes of EKG, under both baseline and challenge conditions (solving anagrams under time constraints), from 40 women residing in a woman's shelter subsequent to domestic abuse, who had completed the civilian PTSD Checklist (PCL-4). The mean PTSD score was 40, a diagnostic criterion. Under baseline conditions, there was a negative quadratic relationship (R^2=0.263, p=0.004, Standardized Beta=0.35) between PTSD scores and Heart Rate Variability (HRV). The relationship between PTSD and HRV was relatively flat for PTSD scores under 40, but then dropped precipitously; consistent with evidence that trauma induces cardiovascular pathology (Soldier's Heart). However, heart rate was negatively correlated with HRV (r=0.605) and positively correlated with PTSD scores (r=0.415), suggesting that the relationship between HRV and PTSD scores may be the result of, and secondary to, reduced inter-beat interval, resulting in an accidental/non-causal relationship between PTSD and HRV. Hierarchical regression analysis revealed a relationship between PTSD scores and HRV when heart rate was controlled for ($r_{partial}$ = -0.302, p=0.062). These relationships remained the same under challenge conditions.
Reports also indicate that reduced HRV is a predictor of future development of PTSD. Given evidence of a bidirectional relationship between PTSD and HRV, path analysis of the present data was used to examine potential causal relationships of Heart Rate and HRV (exogenous variables) on PTSD scores (endogenous variable). Analysis indicated a stronger relationship between HRV and PTSD scores (standardized beta = 0.35) than that between Heart Rate and PTSD scores (standardized beta = 0.21, both directly and via mediation by HRV.
Treating PTSD scores as exogenous, PTSD scores had a stronger putative influence of Heart Rate (standardized Beta = 0.42) than upon HRV (standardized beta = 0.27). But the mediated path from PTSD scores to HRV, via Heart Rate, is weaker (standardized beta = 0.21) than the non-mediated path from PTSD scores to HRV (beta = 0.27), suggesting a direct path from PTSD scores to HRV.

The present data suggests that both HRV and Heart Rate have independent relationships with PTSD scores, which may be di-directional.

Disclosures: R.L. Lloyd: None. **A. McCarty:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.09/Web Only

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: Unconscious re-exposure combined with visual interference enables fearful and traumatic emotion reduction

Authors: *J. HU, Y. WANG, J. GUO, J. DAI;
Zhejiang Univ., Hangzhou, China

Abstract: Direct re-exposure to reminders of major stressors is central to many established treatments for affective disorders, such as prolonged exposure therapy, cognitive reappraisal, and Eye Movement Desensitization and Reprocessing (EMDR). However, the requirement to reexperience these affective events during therapy is inherently aversive to patients, which may lead to premature dropout or even secondary emotional harm. To address this issue, the present study introduces an innovative unconscious re-exposure procedure that subliminally presents visual cues associated with fearful and traumatic memories using a strict visual masking procedure. Two types of visual interference -- bilateral eye movements (BEM) and Transcranial Magnetic Stimulation (TMS) -- were subsequently applied to disrupt the reactivated emotional memory traces. Across three behavioral experiments, unconscious re-exposure combined with BEM significantly reduced both behavioral and physiological responses to fearful memories, with effects persisting for at least one week. These results were replicated in the neural interference task which applied TMS to inhibit activity in the early visual cortices, V1/V2, following either conscious or unconscious re-exposure to cues for traumatic memories. Results not only confirmed the effectiveness of unconscious re-exposure in reducing traumatic memories but also revealed an equal level of effectiveness of unconscious to conscious re-exposure. Moreover, both the behavioral and neural interference studies reported reduced negative feelings during unconscious than conscious re-exposure. Together, these findings suggest a promising approach for attenuating traumatic emotional responses by combining unconscious re-exposure with targeted visual interference. This method may enhance the accessibility and tolerability of treatments for affective disorders, potentially lowering dropout rates while maintaining therapeutic effectiveness.

Disclosures: J. Hu: None. **Y. Wang:** None. **J. Guo:** None. **J. Dai:** None.

Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.10/Web Only

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Title: A sensory cortex origin of flashback memories: integrated evidence from TMS and fMRI

Authors: *J. DAI[1], Z. XU[2], J. GUO[1], J. HU[1], Y. WANG[1];
[1]Zhejiang Univ., Hangzhou, China; [2]KU Leuven, Leuven, Belgium

Abstract: Traumatic memories often intrude into consciousness in the form of vivid sensory flashbacks, inducing psychological influences even long after trauma experience. The origins of these memory intrusions have traditionally been attributed to heightened affective responses and impaired cognitive control, linked to abnormal activity in emotion-processing and prefrontal control networks. However, unlike ordinary episodic memories, flashback memories are predominantly visual and immersive, suggesting a possible role of early sensory cortices in their generation and persistence.To examine this possibility, we combined repetitive transcranial magnetic stimulation (rTMS) and functional magnetic resonance imaging (fMRI) to investigate the causal involvement of early visual cortex in the occurrence and modulation of flashback memories. Using a trauma film paradigm, we induced analogue traumas in healthy participants (N = 48, 32 females), who then received rTMS targeting either the early visual cortex (V1/V2) or the Vertex (control). Compared to controls, rTMS over V1/V2 significantly reduced both the frequency and emotional intensity of trauma-related memory intrusions over the subsequent week. Univariate and multivariate analysis of fMRI data during trauma film encoding revealed that scenes later associated with actual and potential intrusions elicited heightened activation and increased representational similarity across the visual cortex, as well as in the middle frontal gyrus (MFG), a region implicated in cognitive control. Notably, excessive activation in visual cortices and insufficient recruitment of the MFG predicted the persistence of intrusions resistant to intervention. These regions were also re-engaged immediately prior to the spontaneous emergence of flashback memories. Finally, functional connectivity analyses demonstrated that the MFG exerted causal modulation on early visual areas, with the strength of this influence predicting the likelihood of memory intrusions. This MFG-visual cortex pathway operates independently of the traditional MFG-amygdala route, offering a novel mechanism for sensory-based memory modulation.In sum, our findings provide converging evidence that early visual cortices play a critical role in the generation of flashback memories. The demonstrated efficacy of rTMS in reducing these intrusions highlights the potential of targeting sensory cortices for therapeutic intervention in trauma-related disorders.

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Poster

PSTR407: Human Studies: Trauma

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR407.11/JJ8

Topic: H.07. Post-Traumatic Stress Disorder and Other Psychiatric Disorders

Support: German Research Foundation through the Collaborative Research Center SFB1158, projects B04, B09
H. Lundbeck A/S
DZPG German Center for Mental Health, Partner Site Mannheim, Mannheim, Germany.

Title: Daily-life behavioral and neural mechanisms linking adverse childhood experiences of abuse and neglect to affective well-being in a large sample of healthy individuals

Authors: *L. RAOUL[1], O. BERHE[1], A. BENEDYK[1], M. RENZ[1], T. KREMER[1], Y. LIN[2], N. ROYCHOUDHURY[3], A. MOLDAVSKI[1], J. ANDOH[3], A. S. MEYER-LINDENBERG[1], H. TOST[1];
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Abstract: Adverse childhood experiences (ACEs), such as abuse and neglect, are established risk factors for adult mental disorders, likely through their effects on behavioral and neural mechanisms regulating affective well-being. However, most studies have relied on laboratory tasks or self-report questionnaires and rarely distinguished between abuse and neglect.
We used ecological momentary assessment (EMA) to investigate real-time modulators of affective well-being in daily-life in healthy adults and disentangle specific effects of childhood abuse and neglect. A large sample of healthy adults (n = 485) completed an 8-day EMA protocol, reporting affective valence, negative and positive events, and social contact. Participants also completed the Childhood Trauma Scale, NEO-FFI, BDI, and STAI. Regression analyses linked questionnaire scores to the ACEs total score, controlling for demographics. Multilevel models tested whether the ACEs total score moderated the association between daily events and affective valence. Additional models examined the specific effects of abuse and neglect.
Higher ACEs total score was associated with lower extraversion, agreeableness, and conscientiousness, and higher neuroticism, openness, and trait anxiety. In daily life, ACEs total score predicted reduced momentary affective valence and lower sensitivity to positive and negative events, but heightened social affective benefit. Further analyses revealed specific effects of abuse and neglect. Neglect was associated with lower extraversion and agreeableness, while abuse was linked to lower conscientiousness and higher openness. Controlling for abuse, neglect predicted lower momentary affective valence and increased sensitivity to social contact. Conversely, controlling for neglect, abuse did not affect baseline valence but moderated impact of both positive and negative events on valence. Questionnaire results identified both overlapping and distinct associations of abuse and neglect with well-being. EMA reveal differentiated mechanisms: abuse predicted blunted emotional reactivity to environmental stimuli, while neglect was associated with lower baseline affect and heightened social affective

benefit.
These findings underscore distinct behavioral pathways linking ACE subtypes to well-being. Neuroimaging results will be presented, testing whether abuse is associated with reduced structural and functional development of stress/reward circuits and increased development of self-referential networks and. Neglect, conversely, is hypothesized to enhance the development of social reward-processing circuits.

Disclosures: L. Raoul: None. **O. Berhe:** None. **A. Benedyk:** None. **M. Renz:** None. **T. Kremer:** None. **Y. Lin:** None. **N. Roychoudhury:** None. **A. Moldavski:** None. **J. Andoh:** None. **A.S. Meyer-Lindenberg:** F. Consulting Fees (e.g., advisory boards); Daimler and Benz Foundation, EPFL Brain Mind Institute, Fondation FondaMental, Hector II Foundation, Invisio, Janssen-Cilag GmbH, Lundbeck A/S, Lundbeckfonden, Lundbeck Neuroscience Foundation, Neurot. Other; Speaker fees from Ärztekammer Nordrhein, Caritas, Clarivate, the German Society for Neuroscientific Assessment, Gentner Verlag, the State Medical Association BadenWürttemberg, LWL Bochum, Northwell He. **H. Tost:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.01/JJ9

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH/NIDA
Sapienza University of Rome
Ministero dell'Università e della Ricerca
Ministero della Salute Ricerca
European Union – Next Generation EU

Title: A procedure to identify persistent and effort-independent individual differences in preference for heroin over rewarding social interaction

Authors: *G. D'OTTAVIO[1], A. SULLIVAN[2], S. PEZZA[3], M. RUANO[3], J. MODONI[6], I. REVERTE[7], C. MARCHETTI[4], S. ZENONI[4], M. VENNIRO[8], M. S. MILELLA[9], F. BOIX[10], Y. SHAHAM[11], D. CAPRIOLI[5];
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Abstract: Background: In some individuals, opioid use leads to decreased interest in socially relevant rewards. Recent studies showed that after extended-access heroin self-administration

rats strongly prefer social interaction over single unit-dose heroin infusions. We hypothesized that this strong social preference results from access to a suboptimal heroin dose during testing, and individual differences in heroin versus social choice would emerge if rats were given access to their 'preferred' heroin dose.
Methods: In Experiment 1, we trained male rats to lever-press for social interaction, followed by heroin self-administration under continuous-access, no-timeout schedule, which promotes burst-patterned heroin taking. We then tested the rats for choice between single-unit heroin dose and 1-minute full-contact social interaction, or 5-minute heroin-access (sufficient for burst-patterned heroin taking) and 5-minute social interaction. In Experiment 2, we extended the 5-minute access procedure to female rats and tested heroin versus limited-contact (screen-based) social interaction. We also manipulated response requirements (effort) for heroin.
Results: When rats were given a single-unit heroin dose during choice testing, they strongly preferred social interaction. In contrast, when given 5-minute heroin-access, large individual differences in heroin preference emerged. These differences were independent of sex, social-interaction conditions, and effort manipulations. High heroin intake and burst-patterned heroin taking during self-administration, and high heroin seeking during early abstinence predicted individual differences in heroin preference.
Conclusions: Access to 'preferred' heroin doses during the choice tests leads to stable and effort-independent individual differences in heroin preference. This procedure provides a platform to study mechanisms of resilience and vulnerability to opioid addiction.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.02/JJ10

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH/NIDA

Title: Heroin addiction vulnerability in a rat model is associated with distinct patterns of brain activity and connectivity during heroin and social reward seeking: an FDG micro-PET study

Authors: *A. SULLIVAN, G. D'OTTAVIO, J. L. GOMEZ, M. MICHAELIDES, Y. SHAHAM;
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Background: Not all individuals who use opioids develop addiction; only a vulnerable subset transitions to extreme drug use. Heroin addiction is marked by intense craving and a preference for heroin over socially rewarding experiences. We recently established an operant

choice procedure where a subset of rats consistently chooses heroin over social interaction.
Methods: We trained male and female rats to lever-press for social interaction, then heroin self-administration under continuous-access, no-timeout schedule (6-h/day). We then tested the rats for choice between heroin and social interaction. Based on heroin intake, number of burst events, non-reinforced drug seeking on abstinence day 1, and heroin preference, we categorized rats into addiction 'vulnerable' and 'resilient 'subgroups using K-means cluster analysis. During late abstinence (2-4 weeks), we used [^{18}F]Fluorodeoxyglucose (FDG) positron emission tomography (PET) and scanned the rats at three different time, once in their home cage, once after heroin-seeking, and once after social-seeking tests. We used SPM12 to identify voxel-wise group differences and PMOD4.1 to calculate standardized uptake values (SUVs) for connectivity analyses.
Results: Vulnerable rats differed from resilient rats on the heroin-related measures used for cluster analysis, but not social self-administration and heroin or social seeking during late abstinence. The two groups showed distinct patterns of connectivity in all conditions (homecage, heroin seeking, social seeking). Vulnerable rats showed reduced prefrontal cortex activation during heroin seeking and reduced dorsal striatum activation during social seeking.
Conclusions: Vulnerability to heroin addiction in our rat model is associated with altered brain connectivity and distinct patterns of cue-induced activity. These features may serve as addiction biomarkers and intervention targets.

Disclosures: A. Sullivan: None. **G. D'Ottavio:** None. **J.L. Gomez:** None. **M. Michaelides:** None. **Y. Shaham:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.03/JJ11

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA/NIH

Title: Incubation of discriminative stimulus-controlled heroin seeking in rats

Authors: *A. R. HOLMES, O. BERKO, O. NURUDEEN, D. Q. PHAM, M. B. BRENNER, V. A. LENNON, O. R. DRAKE, S. J. WEBER, Y. GERA, H. BONBREST, K. E. SAVELL, J. M. BOSSERT, Y. SHAHAM, B. T. HOPE, R. MADANGOPAL;
Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD

Abstract: Background: We previously reported that rats' response to cocaine-associated discriminative stimuli (DSs) progressively increases (or incubates) during the first 60 abstinence days and persists up to 300 days (Madangopal, *eLife*, 2019). Here we tested if DS-controlled incubation generalizes to heroin.
Methods: We used our trial-based procedure to train rats to self-administer heroin during DS+

trials (heroin available upon lever press; 0.025 mg/kg/infusion; no drug-paired CSs), but not DS- trials (heroin unavailable) in the same session (2 x 3 h/d; 30 each DS+/DS- trials). In Experiment 1, we repeatedly tested rats for DS-controlled heroin seeking (3 h; 30 each DS+/DS- trials; extinction conditions) after 1, 21, 60, 120, 200, 300 and 385 abstinence days. In Experiment 2, we tested contributions of DS+/DS- to heroin seeking after 21 abstinence days (3 h; 15 each *no-DSs*, *DS+*, *DS-*, and *both-DSs* trials; extinction conditions). Results: In Experiment 1, DS-controlled relapse to heroin seeking was higher after 21 and 60 days of abstinence than after 1 day and persisted for up to 385 days. In Experiment 2, responding on day 21 was low during 'DS-' trials, intermediate during 'no-DSs', and 'both-DSs' trials, and maximal during 'DS+' trials. Conclusions: Incubation of DS-controlled drug seeking generalizes to heroin. Additionally, DS+ and DS- independently control expression and suppression of heroin seeking during abstinence. We currently use Oprm1-Cre knockin rats to test the role of mu-opioid receptor expressing neurons in nucleus accumbens, dorsomedial striatum, and thalamic nucleus reuinens in this new form of incubation of heroin seeking.

Disclosures: A.R. Holmes: None. **O. Berko:** None. **O. Nurudeen:** None. **D.Q. Pham:** None. **M.B. Brenner:** None. **V.A. Lennon:** None. **O.R. Drake:** None. **S.J. Weber:** None. **Y. Gera:** None. **H. Bonbrest:** None. **K.E. Savell:** None. **J.M. Bossert:** None. **Y. Shaham:** None. **B.T. Hope:** None. **R. Madangopal:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.04/JJ12

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: Claustrum circuits associated with incubation of oxycodone seeking after voluntary abstinence

Authors: *K. NEGISHI[1], M. PISHGAR[4], H.-L. WANG[2], H. BONBREST[5], J. M. BOSSERT[6], Y. SHAHAM[3];
[1]IRP/NIDA/NIH, BALTIMORE, MD; [3]Behavioral Neurosci. Br., [2]IRP/NIDA/NIH, Baltimore, MD; [4]Natl. Inst. on Drug Abuse, Baltimore, MD; [5]NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD; [6]Behavioral Neurosci., NIH, NIDA, IRP, Baltimore, MD

Abstract: Background: We recently identified a critical role for the claustrum in incubation of opioid seeking after electric barrier-induced voluntary abstinence (Negishi et al. *J Neurosci* 2025). The claustrum sends projections to multiple cortical areas, including anterior cingulate cortex (ACA) and retrosplenial cortex (RSP). We are currently studying the role of these claustral projections in incubation.
Methods: We trained rats (n=18; 8 females) to self-administer oxycodone (6-h/day) for 14 days.

We then induced abstinence by introducing an electric barrier of increasing intensity near the drug-paired lever. On abstinence days 1 and 20, we tested the rats for oxycodone seeking under extinction conditions (no shock, no drug). We injected cholera toxin B subunit (CTb) into ACA or RSP for dual retrograde tracing and used Fos as an activity marker to examine if claustrum-to-ACA and claustrum-to-RSP projections were activated during the relapse (incubation) test on day 20. We also measured Fos expression in ACA and RSP. For controls, we used rats that underwent oxycodone self-administration and electric barrier-induced abstinence but were not tested for relapse before brain extraction.
Results: The rats showed robust incubation of oxycodone seeking (higher lever presses on abstinence day 20 than on day 1). This incubation was associated with increased Fos expression in claustrum and ACA, and in claustrum-to-ACA and claustrum-to- RSP projections, measured by percentage of Fos/CTb double-labeled cells in claustrum.
Conclusions: Incubation of opioid seeking after voluntary abstinence is associated with activation of claustro-cortical projections, suggesting that these projections contribute to relapse vulnerability.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.05/JJ13

Topic: H.08. Drugs of Abuse and Addiction

Support: 1ZIADA000434 YS
K99DA062788 JJC

Title: Effect of experimenter-induced morphine withdrawal on operant social behavior in rats

Authors: *J. J. CHOW[1], E. PILZ[2], K. M. PITTS[3], Y. SHAHAM[4];
[1]NIDA IRP, Baltimore, MD; [2]NIDA, Baltimore, MD; [3]Northwestern, Chicago, IL; [4]Behavioral Neurosci. Br., IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: Opioid addiction is linked to reduced social connections. In preclinical rodent models, withdrawal following experimenter-administered morphine reduces social interaction. We tested whether this effect extends to an operant rat model of social self-administration. Based on the literature, we also tested whether kappa opioid receptor (KOR) antagonist (LY2456302), or serotonin and dopamine reuptake inhibitors (fluoxetine and GBR12909), could reverse opioid withdrawal-induced social deficits. **Methods:** Rats (n = 66; 33 females) were trained to lever-press for 15 s access to a peer (FR1 schedule, 45 min, every other day). In Experiments 1-3, we assessed social self-administration during early (~16 h) and protracted (3-week) withdrawal following various morphine regimens (10-to-80 mg/kg, s.c.,

twice daily; 10-to-80 mg/kg, once daily; or 10-to-40 mg/kg, every-other-day). In Experiment 4, we tested the effects of LY2456302, fluoxetine, and GBR12909 during early withdrawal after every-other-day morphine (0-to-30 mg/kg). **Results:** As in our previous studies, social interaction functioned as a reinforcer. Operant social interaction was reduced during early, but not protracted, morphine withdrawal. None of the tested compounds (LY2456302: 5, 10 mg/kg, s.c.; fluoxetine: 1, 3 mg/kg, i.p.; GBR12909: 3, 10 mg/kg, i.p.) reversed this effect. **Conclusions:** Early, but not protracted, morphine withdrawal decreases operant social interaction. This effect appears independent of KOR, serotonin, or dopamine signaling.

Disclosures: J.J. Chow: None. **E. Pilz:** None. **K.M. Pitts:** None. **Y. Shaham:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.06/JJ14

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: Chronic buprenorphine decreases incubation of heroin seeking and brain activation in Oprm1-Cre knock-in rats

Authors: J. M. BOSSERT, *H.-L. WANG, K. E. CALDWELL, H. BONBREST, M. S. PISHGAR, R. MADANGOPAL, Y. SHAHAM;
IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: We previously found that chronic buprenorphine treatment decreases several opioid relapse-related behaviors in rats. Here, we examined whether chronic buprenorphine also decreases the incubation of heroin seeking during abstinence. We also used the activity marker Fos to identify brain regions activated during incubation testing and to assess the effect of buprenorphine on this activation. Methods: We trained male and female *Oprm1*-Cre rats and their wild-type littermates to self-administer heroin in Context A for 12 days (6 h/day). On abstinence day 1, we tested the rats under extinction conditions in Context B. On day 14, we implanted osmotic minipumps containing saline or buprenorphine (6 mg/kg/day). On day 21, we tested (or did not test, baseline condition) the rats for incubation of heroin seeking in Context B, after which we anesthetized, perfused, and extracted their brains for Fos immunohistochemistry. Results: There were no genotype or sex differences in heroin self-administration or in non-incubated heroin seeking on abstinence day 1. In both genotypes and sexes, chronic buprenorphine decreased incubated heroin seeking on abstinence day 21. Incubation of heroin seeking was associated with increased Fos expression in prefrontal cortex (anterior cingulate, prelimbic, and dorsal peduncular regions) and striatum (dorsolateral and dorsomedial), and this effect was decreased by chronic buprenorphine. Conclusions: Chronic buprenorphine decreases the incubation of heroin seeking, supporting the predictive validity of the rat model. The

inhibitory effect of chronic buprenorphine on incubation was associated with decreased neuronal activity in striatum and cortex.

Disclosures: J.M. Bossert: None. **H. Wang:** None. **K.E. Caldwell:** None. **H. Bonbrest:** None. **M.S. Pishgar:** None. **R. Madangopal:** None. **Y. Shaham:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.07/JJ15

Topic: H.08. Drugs of Abuse and Addiction

Title: Characterizing a μ-Opioid Receptor-Enriched Neuronal Subtype in the Nucleus Accumbens Shell

Authors: *O. R. DRAKE, J. A. HEINSBROEK, J. PETERS, J. J. DAY;
Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Opioid use disorder remains a public health crisis, with high relapse rates and limited therapies that directly target the neural substrates of opioid motivation. Opioids act via mu opioid receptors (μORs) to modulate mesolimbic dopamine circuits, and opioid signaling within the nucleus accumbens (NAc) plays critical roles in the rewarding and motivational effects of opioids. However, μORs (encoded by the *Oprm1* gene) are expressed across numerous NAc cell populations, and the contributions of distinct *Oprm1*-expressing neurons to opioid-mediated behaviors remain unclear. Recent single-cell and spatial transcriptomic profiling studies across species have revealed that *Oprm1* mRNA is unevenly distributed among subpopulations of medium spiny neurons (MSNs). In both mice and rats, a unique population of MSNs in the NAc shell expresses high levels of *Oprm1* and is selectively marked by mRNA for carbohydrate sulfotransferase 9 (*Chst9*). To investigate how this population responds to opioids, we used 10x Genomics Xenium spatial transcriptomics to profile NAc gene expression following voluntary heroin self-administration and naloxone-precipitated withdrawal in C57BL/6 mice. Our analyses validate the enrichment of *Oprm1* in spatially restricted subsets of neurons that share unique transcriptional properties. Analysis of our Xenium dataset and published single-nucleus RNA sequencing (snRNA-seq) data reveals additional genes enriched in Chst9-MSNs, including *Tshz1* and *Tac3* (the rat ortholog of *Tac2* in mice), previously identified as markers of striatal cell types involved in reward and aversion. Ongoing experiments include snRNA-seq and single-molecule RNA fluorescence in situ hybridization (smRNA-FISH) of the rat NAc shell following heroin and naloxone exposure. To enable functional studies, we have generated a Chst9-Cre-tdTomato transgenic rat, and future experiments include electrophysiological recordings from Chst9-MSNs and CRISPR-mediated deletion of *Oprm1* in this population to assess its role in opioid-related behaviors. By identifying and characterizing a novel μOR-enriched MSN subtype in the NAc shell, this work provides new insight into the cell-type-specific mechanisms of opioid reinforcement and may reveal new targets for therapeutic intervention.

Disclosures: O.R. Drake: None. **J.A. Heinsbroek:** None. **J. Peters:** None. **J.J. Day:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

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Program #/Poster #: PSTR408.08/JJ16

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA049787
KFAS

Title: Pallidal Control of Cocaine-Seeking Reinstatement Through a Thalamocortical Circuit

Authors: *H. JANG[1], M. AGUILAR-RIVERA[2], B. LIM[2];
[2]Neurobio., [1]UC San Diego, La Jolla, CA

Abstract: Drug addiction is a chronic, relapsing brain disorder driven by intense cravings that persist even after prolonged abstinence. These cravings can be intensified by cues, stress, or contextual reminders, but a common and potent trigger is re-exposure to a priming dose of the drug. Despite this, the neural mechanisms underlying drug priming-induced reinstatement remain poorly understood. Here, we show that the ventral pallidum (VP) regulates cocaine priming-induced reinstatement through a specific thalamocortical circuit in mice. Using a cocaine self-administration paradigm combined with chemogenetics, we demonstrate that neurons in mediodorsal thalamus (MD) projecting to the anterior insular cortex (aIC)—but not those projecting to the dorsal prefrontal cortex (dPFC)—are critical for cocaine-seeking behavior. Monosynaptic rabies tracing revealed largely distinct upstream inputs to these two MD subpopulations, supporting their divergent functional roles. Notably, the VP forms a well-organized, circuit-specific architecture with distinct thalamocortical pathways. MD neurons that receive VP input and project to either the aIC or dPFC are anatomically segregated and display divergent patterns of collateralization. These two pallido-thalamocortical circuits also exhibit distinct synaptic adaptations following extinction and cocaine priming. Optogenetic manipulation of VP terminals in the MD revealed that these synaptic changes are necessary for cocaine priming-induced reinstatement. Furthermore, modulation of cortical terminals from MD neurons receiving VP input produced similar behavioral effects, confirming that VP's influence on a specific thalamocortical circuit is essential for relapse. Together, these findings uncover a previously unrecognized mechanism through which the ventral pallidum gates drug priming-induced relapse by dynamically modulating a thalamocortical pathway. This work offers mechanistic insight into subcortical regulation of cortical function during relapse and highlights potential therapeutic targets for addiction.

Disclosures: H. Jang: None. **M. Aguilar-Rivera:** None. **B. Lim:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant F32 DA058453
NIH Grant R01 DA053743
NIH Grant R01 MH114990
NIH Grant R01 DA054714
McKnight Foundation

Title: Role of KCNQ2/3 channels in striatal response to dopamine and cocaine conditioned place preference

Authors: *E. T. JORGENSEN[1], N. FITZGERALD[2], J. J. DAY[1];
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Abstract: KCNQ currents are important for controlling neuronal excitability, serving as a brake against hyperexcitable states in neurons. As such, changes in KCNQ channel expression and activity have been implicated in several neuropsychiatric disorders including substance abuse. While KCNQ subunits are encoded by a family of genes (*Kcnq*1 - *Kcnq*5) that form tetrameric channels, most prior work has targeted these channels with pharmacological approaches that lack genetic specificity. Additionally, little is known about subunit-specific contributions to reward circuitry and drug-seeking behaviors. To better understand the function of KCNQ channels in the striatum, we engineered CRISPR sgRNAs targeting the promoters for *Kcnq2* and *Kcnq3* to permit subunit-specific CRISPR activation/interference strategies. To measure the role of KCNQ subunits on electrophysiological activity and dopamine response, we paired CRISPR manipulations with high-density multielectrode array recordings from striatal neurons. Notably, *Kcnq3* knockdown selectively decreased action potential frequency, while overexpression increased frequency. Surprisingly, both overexpression and knockdown of *Kcnq2* increased action potential burst frequency compared to non-targeting *lacZ* controls. Furthermore, *Kcnq3* knockdown decreased firing rate following dopamine stimulation, suggesting a specific role for the *Kcnq3* subunit in dopamine-induced activity. To assess whether KCNQ knockdown altered drug-seeking behavior, we used a cocaine conditioned place preference paradigm following targeted *Kcnq2* or *Kcnq3* knockdown in the adult rat nucleus accumbens. Though all animals showed a distinct preference for the cocaine paired chamber, we found no differences in preference between KCNQ sgRNAs and non-targeting lacZ controls. Taken together, these results demonstrate an important role for KCNQ channels in striatal cell function and dopamine stimulation. Ongoing studies are expanding these results to assess whether the effects of KCNQ channels on cocaine place preference are dose-dependent. These experiments continue to provide insight into how individual KCNQ subunits impact physiological and behavioral responses to dopamine stimulation and cocaine preference.

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

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

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Program #/Poster #: PSTR408.10/JJ18

Topic: H.08. Drugs of Abuse and Addiction

Support: R01 DA053743
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McKnight Foundation Neurobiology of Brain Disorders Award

Title: Multi-omic profiling reveals cell-type specific gene regulatory networks in the rat nucleus accumbens

Authors: *G. M. TWA[1], C. E. NEWMAN[1], R. A. PHILLIPS, III[2], J. J. TUSCHER[3], J. J. DAY[1]; [1]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL; [2]LIBD, Lieber Inst. for Brain Develop., Baltimore, MD; [3]Neurobio., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The accurate, precise, and robust regulation of gene expression is essential for the development and function of diverse cellular identities. This information is encoded within the genome in cis-regulatory elements, such as enhancers and promoters, which mediate the regulatory interactions between transcription factors (TFs) and target genes. Dysregulation of gene regulatory networks (GRNs) in the nucleus accumbens (NAc) has been implicated in the development of substance use disorders (SUDs). The NAc is a crucial region in the mesolimbic dopamine pathway that is essential for reward-related learning and motivation. While research thus far has identified several TFs and chromatin modifiers whose activity in the NAc is altered following drug experience, cell type-specific enhancer activity and overall gene regulatory architecture of the NAc remain poorly resolved. To address this knowledge gap, we collected single-nucleus multi-omic sequencing (paired snATAC-seq and snRNA-seq) datasets to generate hypotheses of cell-type-specific enhancer-driven GRNs (eGRNs) in the NAc. Within our multi-omic data set of ~68,000 NAc nuclei from 23 (Male + Female) rats, we identified 10 transcriptionally defined neuronal and glial cell populations. Across these cell types, we identified 191 activator eRegulons (eGRN components) consisting of transcription factors (TFs), the putative enhancers they bind, and target genes whose transcription they positively regulate. We determined the eRegulons with the highest and most specific activity for each cell type. In microglia, we identify Irf8-driven eRegulons, whose enhancer binding activity has previously been shown to shape microglia-specific transcriptional programs. In MSNs, we find Zeb1-driven eRegulons, a TF whose dysregulation of target genes has recently been associated with cocaine use disorder in the human caudate nucleus. Preliminary analyses also identified Pbx1 and Pbx3 as TFs that target putative enhancer regions and marker genes specific to a unique MSN cluster that expresses high levels of *Grm8*, *Tshz1*, *Oprm1*, and *Chst9* mRNA. Understanding the biological processes these eRegulons regulate, their regulatory syntax, and association with

genes/processes previously implicated in drug-mediated adaptations will be essential for uncovering the genomic mechanisms underlying drug-induced neuroadaptations in SUD.

Disclosures: G.M. Twa: None. **C.E. Newman:** None. **R.A. Phillips:** None. **J.J. Tuscher:** None. **J.J. Day:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Topic: H.08. Drugs of Abuse and Addiction

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NIH R01DA053743
NIH R01DA054714
McKnight Foundation

Title: Transcriptionally defined subpopulations of ventral tegmental area dopamine neurons exhibit distinct electrophysiological properties and response to cocaine

Authors: *N. FITZGERALD[1], E. JORGENSEN[2], J. J. DAY[2];
[1]UAB Neurosci. Grad. Programs, Birmingham, AL; [2]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Substance use disorder is a complex neurobiological disease characterized by a loss of control over drug-taking and drug-seeking behaviors. Drugs of abuse increase dopamine (DA) transmission from ventral tegmental area (VTA) neurons that densely innervate the nucleus accumbens. Using single nucleus RNA sequencing, we previously profiled the VTA and identified unique markers for two distinct subpopulations of DA neurons. *Slc26a7*, a gene that encodes an anion transporter, serves as a selective marker for combinatorial neurons that express genes implicated in glutamate, GABA, and DA neurotransmission. Likewise, the GTP cyclohydrolase member *Gch1* was identified as a marker for canonical DA-only neurons. Here, we used multiplexed fluorescence in situ hybridization to examine whether distinct DA neuron subpopulations respond differently to drugs of abuse. We identified unique induction of the neuronal activity marker *Fos* in *Slc26a7*+ cells in the VTA 1 hour following cocaine experience that was not observed in *Gch1*+ cells, suggesting a difference in response to cocaine between these DA subpopulations. Notably, neither fentanyl nor fentanyl and cocaine co-administration significantly elevated *Fos* mRNA in either subpopulation. To address this distinction between DA subtypes, we designed and generated two novel adeno-associated viruses (AAVs) to label the two cell types independently using a fluorophore and Cre recombinase expressed from cell-type specific promoters (Gch1-Cre-P2A-mCherry and Slc26a7-Cre-P2A-EGFP). Following bilateral VTA injection, 94.00% of *mCherry*+ cells were *Gch1*+ as identified using RNAscope,

and 90.24% of *eGFP*+ cells were *Slc26a7*+ suggesting high targeting efficiency of the two viruses in labeling their respective DA subclass. Using these AAVs, we performed ex-vivo whole-cell patch clamp slice electrophysiology to determine both passive and active properties of these two distinct VTA DA subtypes. While *Gch1*+ cells have properties similar to previously reported tyrosine hydroxylase positive neurons in the midbrain, *Slc26a7*+ cells have a longer time constant and smaller after hyperpolarization potential. Furthermore, *Slc26a7*+ cells have a shorter action potential halfwidth and exhibit longer action potential trains throughout the step protocol, highlighting the ability of the combinatorial cells to maintain firing at higher current input. The observed divergence in membrane properties and action potential properties, alongside differential responses to cocaine, suggests the presence of two distinct subpopulations of VTA DA neurons, each potentially specialized for different roles in input processing.

Disclosures: N. Fitzgerald: None. **E. Jorgensen:** None. **J.J. Day:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

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Program #/Poster #: PSTR408.12/JJ20

Topic: H.08. Drugs of Abuse and Addiction

Support: RS-2024-00332024
25212yakpyeonggi003

Title: Development of molecular-level harm assessment technology for each stage of drug addiction

Authors: C. KONG[1], H. JIN[1,2], J. BAE[1], H.-P. KIM[3], E.-C. LEE[3], M. LEE[4], *H.-I. IM[1,2]; [1]Ctr. for Brain Function, Brain Sci. Institute, Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [2]Div. of Bio-Medical Sci. & Technology, KIST school, Korea Univ. of Sci. and Technol., Seoul, Korea, Republic of; [3]Dept. of Tropical Medicine, Inst. of Tropical Medicine, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [4]Dept. of Life Science, Dongguk University-Seoul, Goyang, Korea, Republic of

Abstract: Significant differences in neural circuitry and molecular-level changes in the brain exist between the early stages of drug use (impulsive use), when drugs are used lightly for pleasure, recreation, etc., and the later stages of drug use (compulsive use), when drugs are used consistently and in large quantities. Current research lacks a comprehensive understanding of the transcriptomic-epigenomic mechanisms underlying drug addiction, highlighting the need for new techniques to assess harmful effects across stages of use and specific brain reward regions. While single-cell multi-omics advances offer promising tools, integrative studies remain insufficient, with most existing analyses providing only superficial insights into molecular and neural changes. Therefore, this study aims to build a standardized big data platform for analyzing epigenetic changes across addiction stages and reward brain regions, assessing individual

differences, and assessing addiction harm. A mouse model was established using a drug self-administration paradigm that encompasses both the impulsive and compulsive phases of addiction. After behavioral characterization, brain tissue was collected from key reward areas and subjected to advanced single-cell multi-omics analysis, including simultaneous transcriptomic and epigenomic profiling. In the compulsive use mouse model, exposure to electric foot shock during drug self-administration after withdrawal was used to categorize the extended model. In this study, behavioral and molecular profiles of specific brain reward circuits established a robust animal model that reliably distinguishes between hypocraving, hypercraving, and compulsive stages of drug addiction. Single-cell multi-omics analyses also revealed stage-specific epigenetic changes and identified novel candidate biomarkers associated with addiction progression. In addition, a model of compulsive use stages was extended to identify individual differences, separating low- and high-compulsive groups. Taken together, through single-cell multi-omics approaches, this study will enable precise, standardized assessment of drug addiction harm and facilitate the discovery of stage-specific biomarkers for early diagnosis and personalized treatment. The resulting data and analytical tools are expected to significantly advance addiction prevention strategies, policy-making, and next-generation drug development, with broad applications across biomedical research and public health.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.13/JJ21

Topic: H.08. Drugs of Abuse and Addiction

Support: K99 DA059608
ZIA DA000467
NIH SIP
NIH CCB

Title: Transcriptional profiling of cocaine relapse ensembles in the rat medial prefrontal cortex to inform drug repurposing strategies

Authors: *K. E. SAVELL[1], R. MADANGOPAL[1], J. M. BOSSERT[2], M. SAGONA[1], A. R. HOLMES[1], O. NURUDEEN[1], O. BERKO[1], D. THOMPSON[1], O. R. DRAKE[1], D. PHAM[1], M. BRENNER[1], Y. SHAHAM[3], B. T. HOPE[4];
[2]Behavioral Neurosci., [3]Behavioral Neurosci. Br., [4]Behavorial Neurosci., [1]NIH, NIDA IRP, Baltimore, MD

Abstract: Background: There are no effective treatments to prevent relapse to cocaine. Cues previously associated with cocaine taking can provoke relapse during abstinence. One strategy to

decrease relapse in rodent models is to disrupt neuronal ensembles encoding drug-cue associative memories activated during relapse tests. However, the cell types comprising the relapse-associated ensembles and how they maintain this association at the molecular level are unknown. To answer this question, we recently developed Multiple**X**ed **Po**pulation **S**election and **E**nrichment single nucleus RNA-**seq**uencing pipeline (XPoSE-seq) to perform targeted snRNA-seq on cocaine relapse ensembles, providing an unbiased screen of ensemble-specific cell types and transcriptional changes during cocaine relapse at the individual animal level. **Methods:** Male and female Fos-mRFP rats were trained to self-administer cocaine (FR1 reinforcement schedule, 0.75 mg/kg/inf. Cocaine infusions paired with a 3.5-s light cue) during twice daily 3-h sessions. Following training and 21 days of abstinence, we tested rats for cocaine seeking (30 min, extinction conditions) and extracted their brains 3 h after test (peak Fos-driven mRFP expression). **Results:** The rats showed reliable cocaine self-administration during training and relapse to cocaine seeking following abstinence. We applied the XPoSE-seq pipeline to identify which cell types comprise the cocaine-relapse ensemble and to characterize the associated cell-type specific transcriptional signatures. We identified distinct clusters corresponding to known cell types in the mPFC (excitatory and inhibitory neurons) that further subcluster into expected layer and interneuron sub-types within mPFC. We used drug repurposing analysis on the transcriptional signatures to identify drugs with compensatory molecular changes, which identified 35 FDA-approved candidates. We are currently testing a subset of these drugs for their effect on relapse to cocaine seeking.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR408.14/JJ22

Topic: H.08. Drugs of Abuse and Addiction

Title: Structural plasticity of rat nucleus accumbens neuronal ensembles after cue-induced cocaine seeking

Authors: *E. UKPONG[1], C. MEJIAS-APONTE[3], D. OLIVARES[2], A. KELLY[1], E. M. HILAIRE[4], L. F. KANE[5], F. RUBIO[6], B. T. HOPE[7];
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Abstract: Learned associations between discrete cues or contexts and a drug are believed to be encoded by sparsely distributed neurons known as neuronal ensembles. Synaptic ensembles are

specific patterns of synapses on these neurons, where input-specific alterations occur during learning to mediate long-term memories. While a novel context exposure induces strong neuronal and synaptic activity, we hypothesize that these morphological changes differ from those seen after cue-induced cocaine seeking or in animals with prior cocaine self-administration training. Dendritic tree and spine morphology may differ between neurons mediating long-term memory and those activated by novel contexts. We examined dendritic spine morphology on medium spiny neurons in the nucleus accumbens (NAc) following cue-induced cocaine seeking. Rats were trained to lever press for cocaine paired with a 3.5-s light cue. Post-training, all rats received intra-NAc injections of AAVs encoding Cre recombinase and Cre-dependent GFP to sparsely label neurons. After 3 weeks in home cages, rats were exposed for 30 min to either the cocaine-paired cue (relapse group), a novel context, or remained in home cages as controls. Brains were collected 60 min post-exposure, processed for Fos immunohistochemistry, and cleared for confocal imaging. GFP-labeled dendritic spines of Fos-positive and Fos-negative neurons were analyzed in 3D using Neurolucida. In distal dendrites, inter-spine interval (ISI) differences were found between Fos-positive and Fos-negative neurons after cue-induced seeking, but not after novel context exposure in naïve or cocaine-experienced rats. Head diameter of mushroom-like spines increased in Fos-positive neurons after novel context exposure in naïve rats, but this was absent in cocaine-experienced rats, suggesting persistent plasticity from learning impairs responsiveness to novelty. However, cocaine cue exposure led to increased spine head diameter in Fos-negative neurons and a higher maturation ratio in these neurons. Thus, in relapse conditions, spine morphology changes involve increased mushroom spine head diameters in Fos-negative neurons and shifts in spine type proportions. Overall, spine remodeling reflects both cocaine history and stimulus specificity (cue vs. novel context).

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Program #/Poster #: PSTR408.15/KK1

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: The role of Grin2c expression in PFC ensemble neurons that encode cocaine conditioned place preference in mice

Authors: *L. A. RAMSEY[1], F. M. HOLLOMAN[1], S. S. LEE[1], S. OH[1], K. E. SAVELL[1], R. MADANGOPAL[1], M. BARBANO[1], M. VENNIRO[3], C. T. RICHIE[2], F. RUBIO[1], B. T. HOPE[1]; [1]NIDA IRP, Baltimore, MD; [2]NIDA IRP, BALTIMORE, MD; [3]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: For decades, treatments for substance use disorders relied primarily on systemic pharmacological treatment strategies, often in conjunction with various forms of behavioral therapy. These methods have been successful for some but often come with intolerable side effects or long-term lack of efficacy. Given that substance use disorder has been characterized as a disorder of maladaptive learning and memory, one critical question is whether there are unique adaptations within neuronal ensembles that support addiction-related learned behavior. We recently developed a pipeline for comprehensive data generation and novel target identification in which we used cocaine conditioned place preference (CPP) in mice to find brain regions strongly activated by recall of a cocaine-associated memory. We uncovered the prefrontal cortex (PFC) as a strongly activated target region. We then performed FACS sorting of PFC neurons after cocaine memory recall to separate neurons into active and non-active neuronal populations. We examined a pre-selected list of target genes with known neurobiological functions. We used quantitative PCR to identify changes in gene expression after cocaine context exposure in several different categories, including genes that encode NMDA receptor subunits, potassium channels, dopamine receptors, and receptor trafficking proteins. We observed that Grin2c, the gene that encodes the NR2C subunit of the NMDA receptor, had a 6-fold increase in expression in response to drug context exposure. We confirmed our finding using RNAscope in situ hybridization. We then used viral delivery into the PFC to perform siRNA-mediated knockdown of Grin2c. Mice that received the Grin2c knockdown virus showed lower cocaine CPP scores after repeated testing compared to mice that received the control virus. Thus, we concluded that Grin2c plays a critical role in encoding cocaine CPP in mice. In future studies, we plan to use this comprehensive pipeline to find and reverse the addicted engram.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

Location: SDCC Halls B-H

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Program #/Poster #: PSTR408.16/KK2

Topic: H.08. Drugs of Abuse and Addiction

Title: A causal role for prefrontal cortex in volitional social memory

Authors: *S. OH[1], Y. DUAN[2], S. LEE[3], F. HOLLOMAN[4], R. MADANGOPAL[5], Y. YANG[6], B. T. HOPE[7], L. A. RAMSEY[8];
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Abstract: The prefrontal cortex (PFC) plays a key role in decision-making, behavioral inhibition, working memory, and social interaction, but its role in operant social learning and memory remains unclear. We previously developed a mouse model of operant social learning to study volitional social self-administration and choice. The aims of this study were first to use the operant social model to identify brain regions activated by volitional social memory, and second to determine the role of the PFC in volitional social memory. For the first aim of our study, we trained female CD1 mice to lever press for access to a social peer mouse. After training, mice were divided into three groups: a "Social memory recall" group in which mice were given a test session during which they lever pressed for access to social cues with no partner present; a "No test" group that remained in the home cage to measure baseline levels of activity without behavioral induction and a "Novel context" group that was placed in an unfamiliar arena to induce high levels of nonspecific neuronal activity. We then extracted brains and performed Fos immunohistochemistry to measure levels of neuronal activity across the 36 different brain regions. We performed hierarchical clustering and graph theory-based modularity analysis to identify 4 modules with correlated activity in these regions. The PFC had high levels of activity that strongly correlated with activity in other brain regions within its module, including the central amygdala and the orbitofrontal cortex. Thus, for the next experiment, we tested the causal role of the PFC in social memory recall. We trained mice as described in the first aim, then co-injected Cre + Cre-dependent caspase virus, or Cre-dependent caspase virus alone (which should remain inert in the absence of Cre) to determine if viral ablation of the PFC impaired social memory recall in the trained mice. Three weeks later, mice underwent a social memory recall test as described above. PFC-lesioned mice showed a reduction in lever pressing for access to social cues, suggesting a deficit in social memory recall. Our future studies will interrogate circuits identified through our social memory recall-induced Fos modularity analysis.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Program #/Poster #: PSTR408.17/KK3

Topic: H.08. Drugs of Abuse and Addiction

Support: ZIA-DA000467
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ZIC-MH002968

Title: Distinct prelimbic cortex ensembles encode response execution and inhibition

Authors: *R. MADANGOPAL[1], Y. ZHAO[3], C. HEINS[4], J. ZHOU[6], B. LIANG[7], G. BARBERA[8], K. LAM[9], L. E. KOMER[10], S. J. WEBER[11], D. THOMPSON[12], Y. GERA[2], D. PHAM[13], K. E. SAVELL[1], B. L. WARREN[14], D. CAPRIOLI[15], M. VENNIRO[16], J. M.

BOSSERT[17], L. A. RAMSEY[18], H. P. JEDEMA[5], G. SCHOENBAUM[19], D.-T. LIN[12], Y. SHAHAM[20], F. PEREIRA[21], B. T. HOPE[22];
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Abstract: Learning when to initiate or withhold actions is essential for survival, requiring the integration of past experiences with new information to adapt to changing environments. The prelimbic cortex (PL) plays a central role in this process, with a stable PL neuronal population (ensemble) recruited during operant reward learning to encode response execution. However, it is unknown how this established reward-learning ensemble adapts to changing reward contingencies, such as reward omission during extinction. Specifically, does the same ensemble adjust its activity to support behavior suppression, or is a distinct ensemble recruited for this new learning? Our data reveal that operant extinction learning recruits a distinct PL Extinction ensemble to support response inhibition, and concerted engagement of both ensembles encodes both ongoing and subsequent context-specific behavior. Using single-cell calcium imaging, we longitudinally tracked PL neurons in rats as they pressed a lever for food rewards (Training), learned to suppress responding upon reward omission (Extinction), and reinstated responding following a non-contingent "priming" pellet (Reinstatement). We trained decoders on individual rats' PL activity patterns to predict trial-wise responses and used a novel *in silico*deletion approach to identify separate PL Training and Extinction ensembles associated with response execution and inhibition, respectively. Critically, both ensembles were re-engaged and maintained their distinct roles during Reinstatement. These findings highlight ensemble-based encoding of multiple, even opposing, learned associations within the same region, demonstrating how selective ensemble recruitment enables behavioral flexibility under changing contingencies.

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Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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NRF-2022R1A2C3008991

Title: Distinct Interneuronal Dynamics Selectively Gate Target-Specific Cortical Projections in Drug Seeking

Authors: *M. JEONG[1], S. BAEK[2], Q. WANG[1], L. YAO[1], J. LEE[1], H. JANG[1], S.-B. PAIK[2], B. LIM[1];
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Abstract: Drug craving persists after prolonged abstinence, posing a major challenge in treating substance use disorders. The ventral medial prefrontal cortex (vmPFC) plays a critical role in impulsivity and decision-making, making it a promising target for mitigating drug-craving by orchestrating downstream brain-wide activity. However, the dynamics of vmPFC sub-circuits during the progression of drug addiction remains unclear. Here, we uncover a circuit-level mechanism by which distinct vmPFC sub-circuits, defined by cell-type-specific interneurons and projection-specific cortical outputs, differentially modulate mesolimbic pathways to drive drug-seeking behavior. Our results reveal that distinct interneuron subtypes display unique activity dynamics and exert selective modulation over projection-specific cortical outputs. Notably, Parvalbumin-positive (PV) interneurons exhibit target-specific synaptic remodeling with pyramidal neurons projecting to distinct downstream targets, which is crucial for modulating mesolimbic circuits and driving persistent cocaine seeking after abstinence. These findings provide compelling insights into vmPFC microcircuit mechanisms underlying substance use disorders.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant DA045764
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NIH Grant DA058653

Title: The Functional Role of the Insula to Medial Prefrontal Cortex Circuit in Distress Tolerance, Drug-Seeking and Affective Behaviors.

Authors: *M. A. SMOAK[1], A. ROMERO[1], T. M. MOSCHAK[2];
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Abstract: Distress tolerance (DT) refers to the behavioral capacity to persist in goal-oriented tasks despite experiencing psychological discomfort. Individuals with low DT and elevated impulsivity are at increased risk for drug-seeking behavior and relapse. Anxiety, often conceptualized as a manifestation of distress intolerance, is also linked to greater substance use. The insular cortex (INS) and medial prefrontal cortex (mPFC) are implicated in addiction-related behaviors, and together they constitute core regions within the salience network—a neural system thought to filter salient stimuli (e.g., drug cues) and orchestrate adaptive behavioral responses. Despite this, the functional role of INS-mPFC circuitry in DT and its connection to impulsivity, anxiety-like behavior, and drug-seeking remains underexplored in preclinical models. We hypothesize that activity in the INS-mPFC pathway during DT performance correlates with impulsivity, anxiety, and drug-seeking tendencies, and that this connectivity is altered following cocaine self-administration (SA). To test this, we utilized a dual-virus approach in Long Evans rats (females: n = 10; males: n = 10), delivering Cre-dependent GCaMP6s into the INS and a retrograde Cre-expressing AAV into the mPFC. A GRIN lens was implanted above the INS for in vivo calcium imaging during behavioral tasks. Following DT assessments, subjects completed elevated plus maze (EPM) testing and underwent cocaine or saline/water SA, followed by an abstinence period and re-evaluation of DT, EPM behavior, and neural activity. Chi-square analyses were used to evaluate differences in activity patterns and group-level effects. Preliminary findings indicate that INS-mPFC connectivity is modulated by DT-related events and exhibits altered neuronal signatures following cocaine SA compared to controls. Notably, we observed sex-specific shifts in DT behavior post-SA. Ongoing work will build on these results to further characterize how this neural circuit contributes to drug-seeking, impulsivity, and anxiety in the context of distress tolerance.

Disclosures: M.A. Smoak: None. **A. Romero:** None. **T.M. Moschak:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Topic: H.08. Drugs of Abuse and Addiction

Support: DA058653
DA045764

Title: The Role of the Prelimbic Cortex (PrL) and Dorsomedial Striatum (DMS) in Costly Drug-Taking and Voluntary Abstinence

Authors: *P. A. FOGEL, L. MORENO, T. M. MOSCHAK;
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Abstract: Cocaine use disorder (CUD) remains without FDA-approved pharmacotherapies, and individuals with substance use disorders (SUDs) often face increasing effort- or punishment-based costs. While both influence drug-taking, they appear behaviorally distinct. To investigate the underlying circuitry that supports cost-based decision-making in SUD, we recorded neural activity from prelimbic (PrL) cortex neurons projecting to the dorsomedial striatum (DMS)—a circuit implicated in cost-benefit evaluation. We injected a retrograde Cre-expressing virus into the DMS and Cre-dependent GCaMP6s into the PrL, allowing projection-specific calcium imaging. Rats were first trained to administer a cocaine or sucrose reward. Then, animals underwent separate bouts of punished or effortful self-administration. Punished self-administration included histamine infusions paired with reward delivery, while effortful self-administration involved various FR schedules. Within the session, the amount of histamine and response requirement (FR) increased systematically during punished and effortful self-administration, respectively. This design produced distinct behavioral phases: pre-conflict (low cost), conflict (balanced cost/reward), and post-conflict (high cost). Preliminary data show that during the conflict phase, when decisions were most difficult, DMS projecting PrL neurons exhibited significantly elevated activity following cue onset, relative to pre- and post-conflict periods. These results suggest that PrL-DMS neurons are preferentially engaged during high-conflict, cost-sensitive decisions. This supports our hypothesis that this pathway tracks motivational conflict during costly cocaine intake and may differentiate vulnerable from resilient phenotypes. Ongoing work will test whether these same neural signatures predict relapse behavior in social choice contexts, offering insight into the neural basis of cost- and socially influenced decision-making in addiction.

Disclosures: P.A. Fogel: None. **L. Moreno:** None. **T.M. Moschak:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant DA058653
NIH Grant DA045764
NIH Grant MD007592

Title: Effects of Optogenetic Stimulation at INS Terminals in PrL on Behavioral Endophenotypes of Male Long Evans Rats Post Cocaine Self-Administration

Authors: *R. SOSA JURADO[1,2], R. POWERS[2], T. M. MOSCHAK[2];
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Abstract: Cocaine use disorder (CUD) is characterized by compulsive consumption, craving escalation, and behavioral endophenotypes such as distress tolerance (persistence in goal-directed behavior amid psychological distress) and impulsivity (lack of restraint and poor decision-making). These endophenotypes are driven by reward and decision-making circuitry, particularly the insula (INS) to prelimbic (PrL) pathway. The INS processes interoceptive and somatoaffective cues, linking internal states to emotional and cognitive responses, while the PrL supports goal-directed behavior, action-outcome associations, and impulse control regulation. Distress tolerance and impulsivity are clinically relevant predictors of relapse. This study tests whether INS-PrL activation influences distress tolerance, impulsivity, and cocaine-seeking behavior in male Long Evans rats undergoing self-administration. Four groups (ChR2/mCherry, cocaine/control) were bilaterally implanted with either p-AAV-CaMKIIa-mCherry-H134-ChR2 or p-AAV-CaMKIIa-mCherry in the INS and optogenetic fibers at the PrL. Animals were trained on a cue-titration paradigm to assess impulsivity and distress tolerance across drug-naïve, short-abstinence, and long-abstinence phases. Cocaine and control groups underwent two weeks of self-administration, followed by extinction (EXT1). After short-abstinence measures, animals underwent long-abstinence extinction testing (EXT2) without access to the operant reinforcer to assess craving incubation. Bilateral PrL optogenetic stimulation (473 nm; 2-3 mW, 20 Hz) was performed after one month of abstinence. Preliminary results (n=9) revealed escalating cocaine intake (n=6), while water groups (n=6) showed stable or decreased consumption. During DT3 stimulation, ChR2 rats showed trending decreases in distress tolerance, while mCherry controls remained stable, supporting INS-PrL involvement in distress-related behaviors. Cue-titration metrics indicated variable effects on impulsivity, requiring further analysis. This study provides insight into the neurobiological basis of cocaine-seeking behavior by targeting the INS-PrL pathway, which integrates interoception, decision-making, and relapse risk. Findings may inform clinical strategies targeting INS-PrL function to improve outcomes.

Disclosures: R. Sosa Jurado: None. **R. Powers:** None. **T.M. Moschak:** None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Program #/Poster #: PSTR408.22/KK8

Topic: H.08. Drugs of Abuse and Addiction

Title: The Prelimbic (PrL) to Dorsomedial Striatum (DMS) Circuit Differentially Encodes Punishment versus Effort in a High-Cost Cocaine Self-Administration Choice Test

Authors: *L. MORENO;
El Paso Community Col., El Paso, TX

Abstract: Substance use disorder (SUD) is marked by continued drug use despite escalating costs, such as increased effort or the risk of punishment. The prelimbic cortex (PrL) and dorsomedial striatum (DMS) are key regions involved in goal-directed decision-making, but it remains unclear whether this circuit differentially evaluates types of cost—such as physical effort and aversive outcomes like punishment. To test this, animals were trained to self-administer cocaine, followed by bouts of punished and effortful self-administration. We used this task to test the hypothesis that the PrL-DMS circuit may differentially encode punishment and effort, reflecting separate neural processes for evaluating aversive versus physically demanding options. We found that PL-DMS activity was significantly elevated in the 5 s period following cue delivery in the high conflict period only, confirming our hypothesis that this pathway is involved in high conflict self-administration. These findings will help clarify how this circuit contributes to cost-based decision-making and may offer insight into mechanisms underlying the persistence of drug use in the face of negative consequences. To address this, we developed a choice procedure in which rats selected between two cost-based options to obtain a reward: (1) a static, punished nose poke cue that delivered a mild fixed histamine catheter infusion upon reward delivery, and (2) an effortful nose poke cue that required a variable number of responses, allowing us to determine the effort level at which the animal values each type of cost (punished or effortful). This design enabled us to examine how animals weigh qualitatively different costs and adapt their behavior accordingly.

Disclosures: L. Moreno: None.

Poster

PSTR408: Addictive Drugs: Neural Pathways, Networks, and Transcription

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant 5R01DA053301-04

Title: Brain networks underlying impulsivity and its links to substance use in early adolescents

Authors: *Y. WENG[1], S. RILEY[1], R. T. CONSTABLE[2], S. YIP[3];
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Abstract: Background: Impulsivity, the tendency to act without sufficient forethought, is strongly linked to risk for substance use initiation (Dalley et al., 2011). While prior research suggests that the neural correlates of impulsivity involve prefrontal cortical and subcortical structures (Merz et al., 2018), most have modest sample sizes, insufficient statistical power and limited focus on adolescents. This study aimed to identify the neural correlates of impulsivity in

early adolescence and their relevance to future substance use using rest and task-based functional connectivity data from the Adolescent Brain Cognitive Development (ABCD) study (Lisdahl et al., 2018). Methods: We analyzed stop-signal task (SST) fMRI data from 3,807 adolescents aged 9-10. Impulsivity was measured via the UPPS-P scale (Whiteside & Lynam, 2001), which assesses five facets: lack of perseverance, lack of planning, sensation-seeking, negative urgency, and positive urgency. We applied connectome-based predictive modeling (CPM) (Shen et al., 2017), a data-driven method, widely used to identify connectivity networks subserving individual differences in cognitive and behavioral traits. CPM analysis was applied to predict each UPPS-P facet from whole-brain connectivity derived from SST fMRI. We also examined whether these networks predicted positive alcohol expectancy at follow-ups, and compared predictive performance between SST, monetary incentive delay task, emotional N-back task, and resting-state fMRI. Results: CPM identified specific connectivity patterns predicting individual differences in UPPS-P facets, with both shared and unique features. Shared correlates spanned medial frontal, sensorimotor, and frontoparietal networks, while each facet showed distinct functional profiles. Although networks related to impulsivity facets involved similar canonical functional networks, the sensation-seeking network was the most distinct. Notably, this network also uniquely predicted positive alcohol expectancies at 2- and 3-year follow-ups. While task-based fMRI is often expected to outperform resting-state fMRI in behavioral prediction, we found that task-based fMRI did not outperform resting-state fMRI in predicting impulsivity traits, aligning with prior findings (Chen et al., 2022). Conclusion: This study identified networks related to individual differences in impulsivity in early adolescents. In particular, the sensation-seeking network at age 9-10 may serve as a prospective neural marker of future substance use risk. Findings enhance understanding of the neural basis of impulsivity and its potential role in early prevention efforts.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

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Title: Observing the localization and relationship of the alpha1-adrenergic receptor, the adenosine 2A receptor, and dopaminergic cells in the ventral periaqueductal gray following chronic ethanol.

Authors: L. KING[1], J. STEPHENSON[1], J. W. BOGENPOHL[2], *D. A. MITRANO[1];
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Abstract: Alcohol use disorder (AUD) is a prevalent public health problem for which there are few effective treatments. Sleep disturbances are highly comorbid with AUD and contribute significantly to disorder progression. Thus, a critical need is to understand the mechanism by which alcohol regulates sleep behavior. Alcohol is well known to reduce sleep latency, leading to its erroneous use as a sleep aid. The initial decrease in sleep latency, however, is followed by increased sleep fragmentation and problems with insomnia in individuals with AUD. Insomnia-related AUD symptoms have been proposed to be linked to relapse. Various neurotransmitters have been implicated in AUD. Stress increases overall norepinephrine (NE) levels, inducing some individuals to consume alcohol. Dopamine (DA) neurons from the VTA have been shown to increase their activity following ethanol exposure, while withdrawal decreases the activity of these neurons. Lastly, adenosine levels increase following exposure to ethanol, enhancing the drug's sedative effects. We recently uncovered a novel pathway by which NE activates DA neurons in the ventral periaqueductal gray (vPAG). When NE activates the α1AR on astrocytes in the vPAG, it increases release of glutamate, which then causes the activity of the DA neurons to increase, producing wakefulness in rodent models. Unpublished data shows that ethanol engages this pathway, providing a novel potential mechanism through which alcohol can paradoxically drive wake behavior. In this project we explored a possible mechanism through which ethanol regulates this vPAG signaling pathway. Which cells express the A_{2A} receptor and the α1AR in the vPAG and does the localization of either the A_{2A} receptor or the α1AR change after alcohol exposure? Sixteen C57BL/6 mice were divided into two groups- ethanol treated (4 male, 4 female) and saline treated (4 male, 4 female). For a period of 6 days, the mice received 3.5g/kg of ethanol (20% in sterile saline v/v) or 0.9% saline, intraperitoneally. On day 7 and 9, mice received ethanol or saline and then were transcardially perfused. Brains were post-fixed and then immersed in increasing concentrations of sucrose. Tissue was embedded and frozen, sliced coronally into 18µm sections and placed on slides. Currently, tissue is being labeled using RNAscope to examine the caudal vPAG for the localization of tyrosine hydroxylase, GFAP, the α1AR, and the A_{2A} receptor. This neuroanatomical study will provide preliminary evidence of alcohol interactions within a key arousal circuit, defining specific paths for therapeutic development to ameliorate sleep disturbances in those with AUD.

Disclosures: L. King: None. **J. Stephenson:** None. **J.W. Bogenpohl:** None. **D.A. Mitrano:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.02/KK11

Topic: H.08. Drugs of Abuse and Addiction

Title: Modulating Alcohol Approach Tendencies and Instrumental Responses Using Counterconditioning

Authors: *A. K. VERMA[1], U. CHIVUKULA[1], M. GARBUSOW[2], N. KUMAR[3];
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Abstract: Automatic alcohol approach tendencies play a significant role in problematic alcohol consumption and have thus become a key target of cognitive and behavioral interventions. However, approaches employed in existing studies have demonstrated limited efficacy in altering these automatic action patterns. Counterconditioning (CC), which establishes competing aversive associations with alcohol-related cues, offers a promising avenue for attenuating alcohol approach biases and promoting adaptive instrumental responses. The present study examined the effectiveness of single-session CC in reducing approach tendencies and enhancing avoidance behaviors while utilizing retrieval cues to generalize intervention effects beyond the immediate training context. Thirty-nine adult alcohol users participated in a within-subject experimental design, completing an Alcohol Approach-Avoidance Task (AAT) and a Pavlovian-to-Instrumental Transfer (PIT) task before and after CC, under electroencephalographic (EEG) recording. Neurocognitive changes were assessed through event-related potential (ERP) components, focusing on centro-parietal P3 (cognitive control) and prefrontal N400 (conflict processing). Participants were classified post hoc into approach- and avoidance-tendency subgroups based on baseline AAT scores. Findings revealed a reduction in alcohol approach tendencies and enhanced pushing instrumental responses following CC in the approach group, particularly when retrieval cues were present. At the neural level, the approach group showed attenuated centroparietal P3 amplitudes during the AAT and reduced right prefrontal N400 amplitudes during both the AAT and the PIT in the presence of alcohol stimuli at baseline, compared to the avoidance group. Interestingly, CC significantly enhanced both P3 and N400 amplitudes during the AAT and PIT tasks, indicating improved controlled processing towards alcohol cues. However, even after CC, neural responses in the approach group remained significantly lower than the avoidance group's baseline responses, suggesting a need for prolonged or supplementary interventions to further enhance avoidance behavior. The pronounced baseline differences and the interventional modulation at the right prefrontal region point to its potential role in shaping alcohol approach behaviors and influencing intervention outcomes. Overall, these findings underscore the utility of retrieval cue-driven counterconditioning for modulating alcohol-related approach biases at both behavioral and neural levels, highlighting its potential as a targeted intervention for maladaptive alcohol use.

Disclosures: A.K. Verma: None. **U. Chivukula:** None. **M. Garbusow:** None. **N. Kumar:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Program #/Poster #: PSTR409.03/KK12

Topic: H.08. Drugs of Abuse and Addiction

Support: UL1TR002529
IU School of Medicine Startup
Stark Neuroscience Research Institute Startup

Title: Role of Parvalbumin-Expressing Neurons in the Anterior Insular Cortex-Dorsolateral Striatum Circuitry in Alcohol Drinking

Authors: *A. NGUYEN[1], B. MUNOZ[1,2];
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Abstract: Alcohol use disorder (AUD) is a chronic, relapsing disorder that affects nearly one-third of adults in the United States. Despite the high prevalence of AUD, currently available pharmacotherapies demonstrate varying efficacy, underscoring the urgent need for more targeted and effective treatment strategies. Our previous studies have indicated that alcohol selectively disrupts glutamatergic inputs from the anterior insular cortex (AIC) to the dorsolateral striatum (DLS), and that *in vivo* activation of glutamatergic AIC-DLS synapses reduces binge alcohol intake in male mice. The AIC directs interoceptive attention to salient cues, while the DLS drives habitual learning, collectively promoting compulsive drinking behavior, a hallmark of AUD. Recently, we identified long-range GABAergic (GABA, γ-aminobutyric acid) projections from parvalbumin-expressing (PV+) neurons in the AIC to medium spiny neurons (MSNs) in the DLS. However, the functional significance of corticostriatal GABAergic synapses in regulating alcohol consumption remains largely unexplored. Here, we found that acute alcohol exposure diminishes GABA release from PV+ AIC synapses, whereas chronic exposure differentially affects MSN subtypes in the DLS. Additionally, we showed that *in vivo* activation of the GABAergic PV+ AIC-DLS circuit increases alcohol consumption in female mice. These findings suggest direct GABAergic PV+ AIC-DLS projections play a critical role in modulating drinking behavior. Given the sparsity of inhibitory corticostriatal synapses, the AIC-DLS circuitry represents a highly specific and promising therapeutic target for AUD.

Disclosures: A. Nguyen: None. **B. Munoz:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Topic: H.08. Drugs of Abuse and Addiction

Support: 5R01AA028861-03

Title: Changes in intrinsic excitability in a population of withdrawal-activated neurons in the medial prefrontal cortex of alcohol self-administering mice.

Authors: *S. RODRIGUEZ[1], J. T. O'BRIEN[2], S. KROENER[3];
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Abstract: Alcohol use disorder (AUD) is linked to impairments of the prefrontal cortex (PFC) that compromise cognitive and executive functions, leading to heightened risk of relapse. In rodent models, activation of the prelimbic (PL) or infralimbic (IL) regions of the PFC are thought to promote alcohol intake or aid the extinction of drug memories, respectively. However, even within these regions, neurons may show heterogeneous responses to alcohol (EtOH) or other reinforcers. Using a transgenic mouse line (TRAP2/Ai9) which permits fluorescent tagging of active populations, we identified PL and IL neurons that were active during withdrawal in mice that self-administered either alcohol or sucrose in daily operant sessions. We conducted whole-cell patch-clamp current-clamp recordings in PL and IL neurons that were either active (Ai9+) or inactive (Ai9-) following an 18-hour withdrawal. While overall response rates for sucrose were significantly higher over 3 weeks of self-administration, mice in the EtOH group showed relative higher response rates vs. baseline during a cue-induced reinstatement session following 5 days of abstinence, suggesting the reinforcing properties of EtOH. IL Ai9- neurons showed no difference in firing rates between groups, whereas Ai9+ cells from alcohol-exposed mice showed increased spike frequencies, suggesting differential, substance-specific modulation of neuronal excitability in the IL region. In the PL we observed no differences in firing rates between cells (i.e. Ai9+ vs. Ai9-) or treatment groups (sucrose vs. EtOH). A different cohort of mice was sacrificed immediately after their final drinking session and again current clamp recordings were performed in PL and IL neurons that were tagged (Ai9+) 5 days prior during withdrawal, as well as cells that were inactive (Ai9-) during that previous withdrawal period. IL Ai9+ cells from alcohol-exposed mice showed no change in spike frequencies when compared to Ai9- cell and sucrose control cells. In contrast, PL Ai9+ cells show decreased excitability when compared to Ai9- and sucrose control cells, indicating a neuronal depression that reverses following abstinence and cue induced reinstatement. Taken together, these finding suggest the existences of a subpopulation of neurons active during withdrawal that become hyperexcitable during a protracted abstinence period and following cue-induced reinstatement. These findings underscore the need for further research to elucidate the specific neurophysiological mechanisms through which alcohol withdrawal can affect relapse.

Disclosures: S. Rodriguez: None. **J.T. O'Brien:** None. **S. Kroener:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH grant R01 AA030594
American Heart Association 25PRE1369483

Title: Effect of acute ethanol on prefrontal cortical excitatory-inhibitory microcircuit interactions

Authors: *Y. KO[1], M. SAUNDERS LUCY[2], R. HUDA[2];
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Abstract: Acute alcohol intoxication impairs cognitive function in part by affecting neuronal activity in the prefrontal cortex (PFC). However, the effect of intoxication on the in vivo activity of single and populations of PFC neurons remains understudied. Local GABAergic interneurons play an important role in orchestrating cortical activity. Whether and how alcohol modulates the in vivo activity of diverse subtypes of PFC inhibitory neurons and interactions between excitatory and inhibitory neurons is not well understood. Previous work from our lab shows that acute ethanol intoxication modulates the activity of a small subset of pyramidal (PYR) cells in the anterior cingulate cortex (ACC) subdivision of the PFC. The fact that the activity of a majority of ACC PYR neurons remained unaffected is surprising, given previous work in ex vivo slices suggesting a largely suppressive effect of alcohol on cortical excitatory cells. We reconcile these differences by hypothesizing that interneurons modulate their activity to maintain net excitatory output following ethanol exposure in vivo. We tested this idea using two-photon calcium imaging of ACC activity in mice given intraperitoneal injections of 0.5, 1.5, 2.0, or 2.5 g/kg of ethanol or saline as a control. We assessed the effects of acute ethanol exposure on the activity of PYR neurons and three inhibitory interneuron subtypes, vasoactive intestinal peptide (VIP)-expressing, somatostatin (SOM)-expressing, and parvalbumin (PV)-expressing neurons. Mice were injected with AAV viruses to express the green genetically encoded calcium indicator GCaMP8s under the control of the synapsin promoter to label all ACC neurons (including the PYR cell population). We used Cre or Flp-dependent AAV viruses expressing GCaMP6s to label interneuron populations. We implanted a chronic window above the ACC region to gain optical access for two-photon calcium imaging. We observed changes in interneuron activity following ethanol exposure consistent with our hypothesis, both at the single-cell and population levels. Additionally, we observed changes in VIP interneuron synchrony following acute alcohol intoxication, as measured through pairwise correlations of simultaneously recorded neurons. PYR neuron activity was largely resilient to alcohol-induced changes at low to moderate doses. Our results provide a detailed account of how ethanol modulates interactions between distinct components of ACC microcircuits and lay the groundwork for a deeper understanding of the impact of ethanol on cortical computations.

Disclosures: Y. Ko: None. **M. Saunders Lucy:** None. **R. Huda:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Topic: H.08. Drugs of Abuse and Addiction

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Title: Social isolation following alcohol consumption suppresses subsequent intake in mice

Authors: *L. BEUGELSDYK[1], E. DECKER RAMIREZ[1], S. SPENCER[1], T. NGUYEN[1], E. HALPERT[1], S. PENDELA[1], J. R. SCHANK[2];
[1]Univ. of Georgia, Athens, GA; [2]Univ. of Georgia Interdisciplinary Neurosci. Program, Athens, GA

Abstract: Continuation of alcohol consumption despite negative consequences is a hallmark trait of individuals with Alcohol Use Disorder (AUD). This behavior can be preclinically assessed using rodent models of aversion-resistant alcohol consumption. Most current models induce aversion by directly pairing alcohol with a negative stimulus, such as footshock or adulteration of the alcohol solution with a bitter tastant. If the subject continues to consume alcohol despite these aversive consequences, it is considered to be aversion-resistant. However, such methods are not optimally translational, as individuals with AUD often face negative consequences that are temporally removed from their alcohol consumption, such as loss of personal relationships following a bout of drinking. To address these concerns, we developed a model in which mice are pair-housed and trained to consume alcohol, then repeatedly socially isolated following alcohol drinking sessions. We used the CD1 strain, as they are more receptive to social isolation than C57BL6/J mice. However, as this strain does not readily consume as much alcohol, we modified a two-bottle choice procedure to include 0.2% saccharin in the alcohol solution, as well as in the water, to stimulate higher levels of drinking. We found that social isolation following alcohol consumption significantly decreased subsequent alcohol consumption, relative to mice that were not isolated (males: $t(10)=2.61$, $p=0.026$; females: $t(14)=2.29$, $p=0.038$). Additionally, once this suppression of intake had been induced, we collected brain tissue following a drinking session to examine differences in neuronal activation between isolated and control mice. Whole brains were perfused, sectioned, and stained for cFos protein as a marker of activation. Multiple brain regions broadly involved in reward and motivation were imaged at 40x magnification. Subsequent quantification revealed that previously isolated mice had increased neuronal activation in the dorsomedial prefrontal cortex ($t(10)=2.74$, $p=0.021$), ventromedial prefrontal cortex ($t(10)=2.66$, $p=0.026$), and nucleus accumbens core ($t(10)=3.52$, $p=0.0055$), relative to control mice. Thus far, neuronal activation has only been examined in males, therefore, current studies are working to examine neuronal activation in females. Additionally, we saw a considerable amount of individual variability within the isolated group, and further analysis is being conducted to determine if individual differences in suppression are correlated with neuronal activation.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIAAA R01AA029403
NINDS T32NS115667

Title: Intrinsic excitability of prelimbic somatostatin neurons across hormonal states following binge alcohol consumption

Authors: *V. NUDELL, N. CROWLEY;
Biol., Pennsylvania State Univ., University Park, PA

Abstract: A robust literature shows sex differences in alcohol consumption and Alcohol Use Disorder among humans, and in rodent models, female C57BL/6J mice consume more alcohol than males when exposed to alcohol via the voluntary Drinking in the Dark (DID) binge drinking model. Previous studies from our lab show prelimbic (PL) somatostatin (SST) neurons modulate an alcohol binge drinking microcircuit, demonstrate basal sex differences in inhibitory current amplitude, and alcohol-induced changes to these currents are sex-dependent. To better understand how PL SST neurons are impacted by estrogen (E2), I investigated intrinsic excitability across the estrous cycle and between sexes following DID with ex vivo slice electrophysiology. Adult SST-Ai9 transgenic mice were used in this study, with animals single housed for 14 days before starting the DID paradigm. Females were grouped by estrous stage representing low E2 (metestrus/diestrus) or high E2 (proestrus/estrus) hormonal states and compared to males. Whole cell current clamp recordings of layer 2/3 PL SST cells were conducted with a potassium gluconate-based intracellular recording solution. Intrinsic excitability measures of resting membrane potential (RMP), action potential (AP) threshold, and rheobase were collected, followed by bath application of vehicle DMSO and E2 to observe the impact on membrane potential in alcohol-exposed animals and water controls. The intrinsic excitability of PL SST neurons according to RMP, AP threshold and rheobase did not vary across the estrous cycle nor with sex. Comparison of membrane potential during vehicle and drug application revealed heterogeneous responses to E2, with some cells depolarizing and others hyperpolarizing from the same animal and/or hormonal group. Ongoing studies are further delineating the interaction between alcohol consumption, biological sex, and PL microcircuits.

Disclosures: V. Nudell: None. **N. Crowley:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Topic: H.08. Drugs of Abuse and Addiction

Support: 5R01AA028145-05

Title: Acute sleep restriction alters future alcohol drinking trajectories in a sex-dependent manner

Authors: *A. ALTAMIRANO-ESPINOZA;
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Abstract: Alcohol is the most commonly abused drug worldwide, and the effects of alcohol on sleep contribute to the development of alcohol use disorders (AUDs). While it is well established that chronic alcohol use leads to disruptions in sleep, less research has investigated the effects of disrupted sleep on initiation and maintenance of alcohol drinking. To investigate this question, we fitted male and female Long Evans rats (n=28,n=31; respectively) with EEG and EMG electrodes to record sleep architecture prior to alcohol exposure, in response to acute 6 hr sleep deprivation, and after 3 weeks of alcohol drinking using the intermittent access 2-bottle choice (IA2BC; 20% v/v) procedure. Recordings were then scored using Somnivore software to identify periods of wakefulness, non-rapid eye movement [NREM] sleep, and REM sleep. In line with previous reports, we found that acute sleep deprivation induced significant changes in sleep architecture. Furthermore, in rats that were not exposed to acute sleep restriction, we observed the expected sex difference of greater alcohol intake in females. However, no sex difference was observed in rats with acute sleep restriction experience, which was largely driven by increased drinking in males. Finally, correlation analyses suggested that sleep patterns immediately prior to alcohol consumption can explain alcohol intake behavior after acute sleep restriction. In conclusion, our results suggest that individual differences in sleep quality may be associated with the propensity to consume alcohol in a sex-specific manner.

Disclosures: A. Altamirano-Espinoza: None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Rhode Island Foundation Medical Research Fund (20210957) to Kristin Scaplen

Title: Alcohol-induced Locomotor Activity in the Fan-Shaped Body of Drosophila melanogaster

Authors: *S. CHARLES[1], K. M. SCAPLEN[2];
[1]Bryant Univ., Providence, RI; [2]Neurosci., Bryant Univ., Smithfield, RI

Abstract: Abstract Alcohol Use Disorder (AUD) is a chronic, relapsing disorder that manifests as problematic alcohol use. It involves disruptions in neural circuits governing reward, motivation, memory, and decision-making and results in enduring preferences, habitual behaviors, and persistent cravings. Alcohol-associated memories are thought to underlie persistent cravings, which reinforce compulsive alcohol seeking. How these memories are disrupted at a systems level remains poorly understood. *Drosophila melanogaster* is a powerful model organism to answer these questions by providing a well-mapped circuitry with lower complexity and sophisticated neurogenetic tools that enable precise manipulation of individual neurons. Previous work in *Drosophila* has identified distinct mushroom body circuits that underlie the acquisition, consolidation, and retrieval of alcohol-associated memories. Interestingly the fan-shaped body (FSB), the largest structure within the central complex in *Drosophila*, was identified as a convergent structure to these circuits. The FSB is also synaptically connected with dopamine circuits important for regulating alcohol-induced locomotor activity. Although the FSB has an established role in regulating arousal, sleep, and memory, its role in the context of alcohol-associated memory and alcohol-induced locomotor activity is unclear. Using an automated group activity monitor called flyGrAM in combination with thermogenetics, we sought to investigate the role of FSB in modulating alcohol-induced activity and the potential overlap in neural circuits for memory and locomotion. Using the temperature-sensitive dominant negative form of dynamin (shi^{ts}) and a collection of freely available split-GAL4 driver lines that target subsets of the FSB neurons we temporarily and reversibly inactivated neurotransmission while flies were exposed to low and moderate doses of alcohol in the flyGrAM. Preliminary data suggest that distinct subsets of dorsal FSB neurons modulate baseline locomotor activity and alcohol-induced changes in locomotor activity. Future work will employ computer vision software to identify discrete behavioral features linked to specific FSB neurons and determine whether the FSB neurons involved in locomotor activity also contribute to alcohol-associated memory. This work extends previous research on how alcohol disrupts behavior and memory formation.

Disclosures: S. Charles: None. **K.M. Scaplen:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Title: Brain Regions Engaged in Binge-Like Drinking in High Drinking in the Dark Line-1 Mice

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Abstract: Drinking alcohol to intoxication (binge drinking) increases the risk of developing an Alcohol Use Disorder (AUD). The nucleus accumbens (NAc) is a brain region important for binge drinking and the development of AUD. We determined which NAc projections and brain regions are engaged in early-stage binge-like drinking using High Drinking in the Dark line 1 mice. We employed a viral retrograde tracer to label NAc projections, a limited access drinking assay to model drinking to intoxication (Drinking in the Dark, DID), c-Fos (an immediate early gene) as a proxy for cellular activity, and whole brain imaging. We found that 4 days of drinking in the dark increased c-Fos in 8 regions projecting to the NAc ($p<0.05$), and decreased c-Fos in 3 regions ($p<0.05$), as compared to water drinking mice. Whole-brain analysis found that following binge-like ethanol drinking, 67 regions had lower ($p<0.05$) and 1 region had higher ($p<0.05$) c-Fos (n=17-21/sex/fluid). This reveals novel and lateralized projections and regions involved in binge-like drinking in a mouse genetic model of risk for drinking to intoxication.
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Disclosures: A.L. Jensen: None. **A.E. Chan:** None. **A.R. Ozburn:** None.

Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: R01AA018734

Title: Activation of GIRK1-containing potassium channels by GiGA1 reduces ethanol-dependent behaviors in mice

Authors: J. TAURA[1], A. MARRAZZO CARRASCO[2], G. RAI[3], C. CONTET[4], ***P. SLESINGER**[1];
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Abstract: Alcohol use disorder (AUD) is a major public health challenge, yet effective pharmacotherapies remain limited. Ethanol modulates neuronal excitability by acting on several

ion channels, including G protein-gated inwardly rectifying potassium (GIRK) channels. Activation of GIRK channels decreases neuronal firing in brain circuits implicated in reward and reinforcement, processes central to the development and maintenance of AUD. Here, we evaluate the efficacy of GiGA1, a selective activator of GIRK1-containing channels, as a novel therapeutic candidate for AUD. Using conditioned place preference (CPP) and voluntary ethanol consumption paradigms (2-bottle choice drinking-in-the-dark; 2BC-DID) in mice, we demonstrate that GiGA1 robustly prevents ethanol-CPP in both sexes, mitigates ethanol-induced locomotor stimulation in females, and significantly reduces voluntary ethanol intake and blood alcohol concentration without affecting water consumption. By contrast, Baclofen, which activates GIRK channels through $GABA_B$ receptors coupled to G proteins, reduces ethanol intake but fails to prevent ethanol-CPP, highlighting GiGA1's broader therapeutic efficacy. Brain-wide cFos mapping reveals that GiGA1 effectively counteracts ethanol-induced neuronal activation in critical AUD-related circuits, including the central amygdala (CeA), paraventricular nucleus of the thalamus (PVT), paraventricular nucleus of the hypothalamus (PVN), and Edinger-Westphal nucleus (EW). Together, these findings suggest activation of GIRK1-containing channels could be a promising new therapeutic approach for treating AUD.

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Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

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Program #/Poster #: PSTR409.12/KK21

Topic: H.08. Drugs of Abuse and Addiction

Support: R01AA030619

Title: Characterizing adolescent alcohol's effect on corticothalamic neurotransmission in a Latrophilin-3 mouse model of ADHD

Authors: *S. G. GAKARE[1], F. MAXWELL[2], H. MEJIA GOMEZ[2], M. SALLING[3];
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Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a heritable neurodevelopmental disorder characterized by inattention, hyperactivity, and impulsivity. Individuals with ADHD are at an increased risk of developing alcohol use disorder (AUD), particularly when alcohol consumption begins during adolescence—a critical period of heightened neural plasticity and vulnerability. Genetic and neuroimaging studies suggest overlapping mechanisms between ADHD and AUD, including delays in frontal cortex maturation and shared risk genes such as Latrophilin 3 (LPHN3), a synaptic adhesion G-protein coupled receptor involved in the

development of glutamatergic synapses. However, the interactions between adolescent alcohol use and ADHD-linked genetic vulnerabilities and their impact on frontal corticothalamic (CT) circuits remain poorly understood. In this study, we employed a conditional *Lphn3* knockout (Lphn3 cKO) mouse model to investigate how exposure to alcohol during adolescence affects CT function. *Lphn3* was deleted from the medial prefrontal cortex (mPFC) of male and female mice (wild type, heterozygous, and mutant) using AAV8-CaMKIIa-CreGFP at postnatal days (PND) 25 to 28. The mice then underwent voluntary alcohol consumption (2-bottle choice) from PND 30 to 60, followed by behavioral testing (alcohol consumption, anxiety, recognition memory, and working memory). CT neurons that project to the mediodorsal thalamus were labeled using AAVrg-Syn-FLEX-TdTomato, and whole-cell patch-clamp electrophysiology was conducted to assess excitatory transmission and intrinsic properties. While adolescent alcohol consumption did not differ by genotype, adult mutant mice consumed more alcohol than wild type and heterozygous mice. Behavioral characterization indicate a near significant interaction of alcohol and genotype on object in place recognition task. Electrophysiological recordings indicate that both Lphn3 deletion and adolescent alcohol exposure independently showed a trend of reduced excitatory synaptic transmission in CT neurons while application of a D1R agonist increased excitatory synaptic transmission. These findings further our understanding of how genetic risk factors and adolescent alcohol exposure converge to affect CT circuitry and identify selective D1R agonism as a potential therapeutic strategy for cognitive dysfunction related to ADHD and AUD.

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Poster

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Title: Ethanol exposure in adolescent female mice induces behavioral deficits and decreases serotonin and microglial marker expression in the raphe nuclei

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Abstract: Heavy alcohol consumption in adolescence increases the risk of poor health outcomes in adulthood, including anxiety, depression, and substance use disorders. Evidence also suggests

that exposure to alcohol can predispose individuals to chronic pain disorders and can exacerbate preexisting conditions. Previously, our lab explored the effects of adolescent alcohol exposure in male mice on pain thresholds, neuroinflammation, and depressive- and anxiety-like behaviors. Recent studies, however, have highlighted key sex differences in how pain is experienced, the body's response to pain, and pathological susceptibility to pain. The goal of the current study was to further our previous work by examining the effects of chronic adolescent exposure to ethanol (AIE) on pain-related behavior changes and neurological changes in the raphe nuclei (RN) circuits of female adolescent mice. Female C57BL/6 mice were exposed to a two-bottle choice paradigm for four weeks beginning on postnatal day 25. AIE mice were given access to one bottle of 20% ethanol and one bottle of tap water on Monday, Wednesday, and Friday and tap water only all other days. Control mice had access only to tap water. Ten mice were in each group as estimated by power calculation. Two to three weeks into withdrawal, and after cessation of AIE, mice were ran through pain testing, open field test, and social interaction, or were perfused and had brains extracted for immunohistochemistry. AIE was found to produce long-lasting hyperalgesia in adult female mice after withdrawal from alcohol. This was seen as a reduced thermal and mechanical pain threshold in the Hargreaves and Von Frey assays, respectively ($t_{18} = 3.644$, $p < .01$; $t_{18} = 3.644$, $p < .01$). AIE mice also had impaired social behavior and spent significantly less time interacting with a stranger mouse than did control mice ($F_{1,36} = 5.968$, $p < .05$). AIE also led to decreased serotonin (5-HT) expression in the median raphe nucleus (MRN) ($t_{18} = 2.922$, $p < .01$) and the raphe magnus (RMg) ($t_{17} = 3.092$, $p < .01$) as well as decreased TPH2 expression in the RMg ($t_{15} = 2.176$, $p < .05$). AIE decreased expression of microglial markers throughout the RN, with decreased CD68 expression in the MRN ($t_{18} = 3.061$, $p < .01$) and RMg ($t_{16} = 3.715$, $p < .01$), decreased P2Y12 expression in the MRN ($t_{18} = 3.104$, $p <.01$), and decreased IBA1 expression in the dorsal raphe nucleus ($t_{17} = 2.196$, $p < .05$) and MRN ($t_{17} = 2.464$, $p < .05$). All analyses were performed by a technician blind to the experimental groups. Results suggest that 5-HT neuron loss and microglial loss could result from sustained adolescent alcohol intake in female mice and could lead to persistent hyperalgesia and social development seen in adulthood

Disclosures: G. Bierlein-De La Rosa: None. **N. Balasubramanian:** None. **K.M. Khan:** None. **J. Reinhardt:** None. **C. Marcinkiewcz:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.14/LL1

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant AA030193
NIH Grant AA019967

Title: The role of prelimbic cortex in mechanical allodynia induced by adolescent alcohol exposure

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Abstract: Neuronal circuitry in the medial prefrontal cortex undergoes significant refinement during adolescence, including maturation of connectivity between the basolateral amygdala (BLA) and the prelimbic (PrL) cortex. Insults occurring during adolescence, such as repeated alcohol use, alter the normal development of this circuitry and its associated cognitive functions. Recent research has implicated BLA inputs to both PrL neurons projecting to the periaqueductal gray (PrL-PAG neurons) and PrL parvalbumin interneurons (PVINs) in regulating pain-related behaviors. Adolescent intermittent ethanol (AIE) exposure induces mechanical allodynia and alters both the intrinsic excitability of PrL PVINs and PrL-PAG neurons and the excitatory/inhibitory balance at BLA inputs onto these neurons. To investigate whether AIE-induced changes in PrL PVIN and PrL-PAG neuron activity contribute to AIE-induced mechanical allodynia, chemogenetics were used to modulate the activity of these neuronal populations. Adolescent PV-Cre rats and their wildtype (WT) littermates were exposed to ethanol using a passive vapor exposure paradigm for four weeks. Viral vectors were then used to express DREADDs or an mCherry control in either PrL PVINs (PV-Cre rats) or PrL-PAG neurons (WT rats). Four weeks post-infusion, rats were habituated to experimental procedures. Next, they received deschloroclozapine (DCZ, 0.3 mg/kg, ip) and were assessed for mechanical and thermal sensitivity using von Frey and Hargreaves tests. Finally, a formalin challenge was administered (DCZ followed by intraplantar formalin [50 uL, 2.5%]), and nocifensive behaviors were recorded. The following day, formalin-induced place aversion was measured. Preliminary findings suggest AIE enhanced formalin-induced place aversion. Moreover, increasing PrL-PAG neuron activity in Air control rats recapitulated the AIE-induced increase in place aversion. These results suggest changes in PrL-PAG neuron excitability contribute to heightened pain sensitivity following adolescent ethanol exposure.

Disclosures: J.D. Obray: None. **M.D. Scofield:** None. **L. Chandler:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.15/LL2

Topic: H.08. Drugs of Abuse and Addiction

Support: R21-AA14010

Title: The Course of Neuroendocrine Healing With Abstinence In ICD-11 Alcohol Dependence

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Abstract: Background: A lifetime alcohol dependence (AlcD; ICD-11) diagnosis occurs in 20%-50% of patients in public and university general hospitals. The estimated adult population rate in the US ranges from 7% to 10%. Subtle early brain healing processes may involve changes in the neuro-endocrine stress system that can affect both cognition and decisions about treatment options. Method: To test whether neuro-endocrine healing occurs in frequent, heavy drinkers, this study 1) compared heavy drinking AlcD test subjects (n=16) with light drinking control subjects (n=15) at baseline and then 2) assessed the test subjects prospectively at three, and six months of supervised, disulfiram assisted abstinence. The subjects provided diurnal salivary cortisol samples on waking, waking +30 minutes, noon and 4 PM for each of the follow-up time points. Significance required alpha = 0.05. Results: Baseline test group cortisol means were significantly higher than control levels ($p < 0.04$ to <0.003) with a notable absence of the morning cortisol response. The Three-Month average diurnal curve suggested an exaggerated morning response ($p< 0.038$), while the Six-Month average curve approximated the baseline control values ($p>0.05$). Conclusions: These data indicate that neuro-endocrine healing occurs with abstinence but is gradual. Healing may take up to six months to reach its full potential. This suggests the possibility that 1) neuro-endocrine effects may impair complex cognition and decision making early in the healing course and 2) neuro-endocrine healing late in the course may account for the often unrealistic sense of well-being that occasions alcohol relapse. Focused treatment at these junctures may serve to expedite healing and add to the likelihood of sustained remission.

Disclosures: T. Beresford: None. **P.J. Ronan:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.16/LL3

Topic: H.08. Drugs of Abuse and Addiction

Support: VA Grant I01 CX001558
NIDA R21 AA020039
VA IK2 CX001790
VA I01 CX002573
NIDA P50DA018165

Title: Multimodal imaging in alcohol use disorder

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[6]Oregon Hlth. and Sci. university, Portalnd, OR; [7]Psychiatry, Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Introduction:
Alcohol Use Disorder (AUD) causes widespread loss of cortical brain volume and damage to white matter tracts connecting affected cortical regions. We investigated the relationship of cortical volume loss to disturbances in white matter integrity in 71 adult subjects with AUD and 89 healthy controls. We hypothesized that reduced cortical thickness and white matter integrity would primarily affect regions in the executive control (ECN) and salience attribution (SAN) networks.
Methods:
MRI data was acquired on a 3 Tesla (T) Siemens Magnetom Prisma scanne. T1-weighted anatomical images and two HARDI scans were acquired. We extracted cortical thickness and measures of sub-cortical volumes using FreeSurfer with a standard pipeline. We used Random Forest with Recursive Features Elimination (RF-RFE) to identify cortical regions that best differentiated AUD and CS. We calculated fractional anisotropy differences between the groups and used obtained probabilistic tractography among a set of 13 pre-selected nodes with white matter connections that were predicted to differ between groups.
Results:
The RF-RFE classifier identified 29 regions that appeared in > 70% of the classification trees. Comparison of these regions with a multivariate general linear model with age as a covariate was significant (Wilks' $\Lambda = 0.7912$, $p < 0.001$). Regions in the ECN and SAN, as well as the Default Mode Network, differed between the two groups. TBSS found higher mean FA values (corrected for multiple comparisons) in control subjects compared to AUD primarily along several white matter tracts: L/R anterior thalamic radiation, L/R inferior fronto-occipital fasciculus, temporal part of the L superior longitudinal fasciculus, L corticospinal, L inferior longitudinal fasciculus, L uncinate fasciculus, and the forceps minor. Probabilistic tractography calculated for the a priori node pairs found 8/13 showed significantly less connectivity in CS relative to AUD, 4 pairs showed trend differences and two pairs were not different.
Conclusions:
These results reinforce the concept that chronic alcohol abuse results in loss of cortical thickness and the associated white matter integrity between regions suffering volume loss. These findings likely result from a combination of cortical neuronal loss, loss of cortical synapses and loss or damage of projection axons. Correlation of tractographic deficits in AUD with regional cortical volume loss strongly suggests damage to projection axons and their cortical cell bodies.
The contents of this paper do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Disclosures: W.F. Hoffman: None. **L. Dennis:** None. **H. Mccready:** None. **S. Swain:** None. **E. Rigales:** None. **J. Nagum:** None. **D. Smith:** None. **M. Kohno:** None.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.17/LL4

Topic: I.04. Executive Functions

Support: NIH: RO1AA025337 (JB, PI)

Title: Acute alcohol intake alters network topology in occipital, frontal, and temporal cortices compared with placebo

Authors: *L. A. BIESSENBERGER (DAVIS)[1], B. STENNETT-BLACKMON[3], S. NIXON[4], A. CUSHNIE[2], J. BOISSONEAULT[1];
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Abstract: Alcohol intake disrupts the function of numerous brain regions involved in cognition and sensory processing. Most neuroimaging studies of acute alcohol effects focus on changes in the magnitude of functional connectivity between brain regions. However, graph theory analysis allows additional inference about how alcohol affects the role of each region of interest (ROI; node) within brain networks, as well as the structure of brain networks more broadly. In this study, healthy individuals (n=107, 21- 45 yrs, 61 women) consumed alcohol (0.08 g/dL target BrAC) or a placebo drink (sugar-free lemon lime soda) in 2 double-blinded sessions. Resting state MRI was acquired with a Siemens Prisma 3T scanner 30 min after consumption. We used general linear models to identify the effects of alcohol vs. placebo on graph theory outcomes in a network of 106 cerebral ROIs (p_{FDR}<.05) using the CONN toolbox. The ROI-ROI cost threshold for significant edges, which sets the sparsity of the graph, was defined as k>.25. Results revealed alcohol significantly perturbed global efficiency (E_{glo}) local efficiency (E_{loc}), closeness centrality (C_{CLO}), degree (D), clustering coefficient (C_p), and mean path length (L_p) for nodes in the occipital, frontal, and temporal cortices. Alcohol significantly decreased E_{glo}, which reflects the degree of connectedness of a region with the rest of the brain, in several occipital nodes and increased E_{glo} for nodes within the frontal and temporal cortex. Alcohol also increased E_{loc} across the network (b = 0.01, p_{FDR} = 0.0115), as well as in specific nodes in the temporal and frontal cortices (b = 0.03 - 0.05, p_{FDR} = 0.015 - 0.017). An increase in E_{loc} indicates an increased weight of information transfer with adjacent nodes. Alcohol significantly increased D in frontal and temporal regions (b = 5.22 - 6.13, p_{FDR} = 0.0006 - 0.04). D reflects the number of edges a node has with adjacent nodes. Alcohol decreased C_{CLO} (b = 0.03 - 0.04, p_{FDR} = 0.031 - 0.003) and increased L_p (b = 0.09 - 0.24, p_{FDR} = 0.047 - 0.005) in parietal and occipital regions as well at the network level (C_{CLO} b = -0.01, p_{FDR} = 0.036; L_p b = 0.04, p_{FDR} = 0.021) compared with placebo conditions. A decrease in C_{CLO} reflects a decrease in the influence of the region on other local nodes, while increased L_p (i.e., the number of edges connecting a node with other nodes in the network) is consistent with greater resource use to support information transfer and less efficient communication (E_{loc}). Together, results show that acute alcohol intake significantly affects cerebral network topology, with specific effects in the occipital, frontal, and parietal cortices.

Disclosures: L.A. Biessenberger (Davis): None. **B. Stennett-Blackmon:** None. **S. Nixon:** None. **A. Cushnie:** None. **J. Boissoneault:** F. Consulting Fees (e.g., advisory boards); Tryp Therapeutics.

Poster

PSTR409: Alcohol: Neural Circuits, Behavior, and Imaging

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR409.18/LL5

Topic: H.08. Drugs of Abuse and Addiction

Title: The effects of alcohol on pain-related brain connectivity during a heat-pain task and resting state fMRI

Authors: *B. FAYZULLOBEKOVA[1,2], K. B. WELDON[3], R. J. HERMOSILLO[4], J. BOISSONEAULT[5], D. A. FAIR[3];
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Abstract: The recently identified SCAN (socio-cognitive, affective, and nociceptive) network is an association system interdigitated in motor cortex, overlapping with cortical and subcortical pain centers. This overlap suggests SCAN plays a role in modulating pain. In this study we investigated the effects of alcohol on functional connectivity during thermal pain and rest, using previously collected data on alcohol analgesia. In the original study, 110 healthy participants (n = 61 female, age = 25.87 ± 4.32 years) completed two counterbalanced fMRI sessions. Prior to scanning participants consumed either (1) alcohol (≈0.08 g/dL blood alcohol) or (2) a placebo beverage. Each scanning session included a structural T1, a 9-min resting-state run, and three 6-min task fMRI runs with painful heat applied to the foot for 10 s in 7 blocks. MRI data were preprocessed with fMRIPrep (v24.0.1). Denoised functional derivatives were generated with XCP-D (v0.9.0; Mehta et al., 2024). After quality control, 96 participants remained for further analysis. Dense functional connectivity matrices were generated from BOLD data, preserving individual network patterns. Group-level averages were used to assess consistent alcohol effects on pain-related brain connectivity. In an initial analysis, we conducted a 2×2 repeated-measures ANOVA to examine the effects of drug (alcohol vs. placebo), task (pain vs. rest), and their interaction on functional connectivity across major brain networks. In brief, there was increased connectivity during pain across drug conditions in SCAN ($p = 0.048$), Default Mode Network (DMN; $p < 0.001$), and dorsal Somatomotor (SMd; $p = 0.016$). SCAN and Cingulo-Opercular (CO) networks showed no significant effect of drug condition or interactions ($p > 0.05$). These findings provide additional evidence for the involvement of SCAN in pain modulation but do not necessarily implicate SCAN in alcohol-induced analgesia.

Disclosures: B. Fayzullobekova: None. **K.B. Weldon:** None. **R.J. Hermosillo:** None. **J. Boissoneault:** None. **D.A. Fair:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.01/LL6

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA DA059646

Title: Gpr83 antagonism enhances morphine induced analgesia and reduces reward-related behavior in rodents

Authors: *A. YADAV[1,2], A. K. FAKIRA[3];
[1]Biomed. Sci., Rowan Univ., Camden, NJ; [2]Biomed. Sci., Cooper Med. Sch. at Rowan Univ., Camden, NJ; [3]Cooper Med. Sch. at Rowan Univer, Glassboro, NJ

Abstract: GPR83 Antagonism Enhances Morphine Induced Analgesia and Reduces Reward-Related Behavior in Rodents **Anubhav Yadav and Amanda K. Fakira Department of Biomedical Sciences, Cooper Medical School of Rowan University, Camden, NJ, 08003**
Background: Chronic morphine use is limited by adverse effects such as tolerance, addiction, and respiratory depression. Current treatments inadequately address these issues. GPR83, an orphan G-protein coupled receptor, is expressed in the periaqueductal gray (PAG) and nucleus accumbens (NAc), brain regions central to pain and addiction. We hypothesized that GPR83 antagonism could enhance opioid analgesia while reducing addiction-related behaviors.
Methods: Using a rodent model, we administered the selective GPR83 antagonist CPD25 in combination with morphine. Analgesic response was measured with the Hargreaves test (paw withdrawal latency), and addiction-related behavior was assessed via conditioned place preference (CPP). Planned studies will quantify dopamine levels in the NAc and GABA in the PAG to explore neurochemical mechanisms. **Results:** Co-administration of CPD25 with morphine significantly increased paw withdrawal latency compared to morphine alone ($p < 0.01$), indicating enhanced analgesia. Additionally, CPD25 reduced morphine-induced CPP ($p < 0.05$), suggesting decreased reward behavior. These findings support a modulatory role of GPR83 in opioid signaling, potentially through interactions with the mu-opioid receptor (MOR).
Conclusion: GPR83 antagonism improves morphine's analgesic efficacy while reducing its addictive potential in vivo. These results highlight GPR83 as a promising therapeutic target to mitigate opioid-related side effects. Ongoing work will clarify the neurochemical basis of GPR83-MOR interactions and further define its clinical relevance

Disclosures: A. Yadav: None. **A.K. Fakira:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.02/LL7

Topic: H.08. Drugs of Abuse and Addiction

Support: The University of Queensland- Australian Government Research Training Program

Title: Bivalent Peptide Agonists Targeting Peripheral Kappa and Nociceptin Opioid Receptors for Pain Management

Authors: *M. HABIB, D. SAIFUDDIN, M. ROBITAILLE, P. CABOT;
Sch. of Pharm. and Pharmaceut. Sci., The Univ. of Queensland, Woolloongabba, Australia

Abstract: The opioid crisis remains a global health concern, intensified by the adverse effects such such as dependence, tolerance, and addiction associated with opioids acting in the central nervous system (CNS). Endogenous opioid peptides like Dynorphin (Dyn) and Nociceptin/Orphanin FQ (N/OFQ), which selectively activates kappa (KOPr) and nociceptin (NOPr) opioid receptors, offer peripherally restricted analgesia with reduced CNS adverse effects. However, their clinical potential is limited due to rapid enzymatic degradation. To address this, bivalent constructs 'DyNo', linking potent fragments of Dyn (Dyn1-7) and N/OFQ (N/OFQ1-13) via a stable linker, were developed to enhance stability, potency, and receptor targeting.

The bivalent constructs were synthesized using solid-phase peptide synthesis. Functional assays were conducted using Chinese hamster ovary (CHO) cell lines stably expressing the KOPr, NOPr, and co-expressing both receptors, i.e., NOPr/KOPr. Forskolin (10 µM) was used to stimulate cAMP production in the cell lines, and agonist-mediated inhibition of cAMP was measured using the Revvity AlphaScreen® cAMP assay. N/OFQ1-13 and U50,488H were positive controls for NOPr and KOPr activation, respectively. Concentration-response curves were generated to determine the half-maximal inhibitory concentration (IC_{50}) of DyNo523. All statistical analysis was conducted using GraphPad prism version 10.1. Unpaired t-tests or one-way ANOVA were employed, as appropriate, based on the data distribution and experimental groups.

DyNo523 demonstrated comparable potency to NOPr selective agonist N/OFQ1-13 in CHO-NOPr (IC_{50}: 7.87 ± 0.71 nM vs 2.45 ± 0.47nM; n=4; P>0.05, t-test) and to KOPr selective agonist U50, 488H in CHO-KOPr cells (IC_{50}: 9.21 ± 2.36 nM vs 3.67 ± 1.56 nM; n=4; P>0.05, t-test). In CHO-NOPr/KOPr cells, DyNo523 exhibited a biphasic response indicative of dual-receptor engagement and yielded IC_{50} values of 1.47 ± 0.61 nM (DyNo523), 2.93 ± 0.35 nM (U50,488H), and 0.41 ± 0.14 nM (N/OFQ1-13) (n=5; *P*>0.05, ANOVA).

DyNo523, a bivalent contruct, retains the potency and efficacy of its monovalent controls in modulating NOPr and KOPr signaling. The comparable cAMP inhibition profiles suggest successful dual engagement of opioid receptors by this bivalent construct. Selected bivalent constructs will undergo further evaluation for metabolic stability across various physiological media in future studies. These findings support further development of bivalent constructs as promising therapeutics for peripheral pain management with reduced CNS adverse effects.

Disclosures: M. Habib: None. **D. Saifuddin:** None. **M. Robitaille:** None. **P. Cabot:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.03/LL8

Topic: H.08. Drugs of Abuse and Addiction

Support: The University of Queensland Graduate School Scholarships Research Training Program

Title: The novel bivalent peptides based on beta-endorphin analogues target the mu opioid receptor.

Authors: *Y. WANG, D. SAIFUDDIN, K. TUPALLY, H. PAREKH, P. J. CABOT;
Sch. of Pharm. and Pharmaceut. Sci., The Univ. of Queensland, Brisbane, Australia

Abstract: Introduction: Pain represents a global clinical problem, and opioids are considered an important treatment option by the World Health Organisation. However, adverse side effects, such as tolerance and addiction, have led to serious safety risks for patients on opioid treatment. Peptides based on endogenous opioid peptides (EOP) are considered a possible alternative treatment for pain, as they largely avoid adverse effects. There are three major opioid receptors: the mu, delta and kappa opioid receptors (MOPr, DOPr, KOPr, respectively). The common opioids, like fentanyl, all primarily target the MOPr to relieve pain. β-endorphin 1-31 (BE1-31) is a potent analgesic EOP selective targeting MOPr. β-endorphin 1-7 (BE1-7) is a functional fragment of BE1-31. This study synthesised and evaluated a series of bivalent peptides based on parental monovalent BE1-7 for their pharmaceutical potency and efficacy. Methods: Solid-phase peptide synthesis (SPPS) was used to synthesise a panel of peptides. Opioid peptide-induced inhibition of the signal molecules cyclic adenosine monophosphate (cAMP) is determined to evaluate efficacy and potency in HEK293 cells overexpressing MOPr. The half maximal inhibitory concentration (IC_{50}) and statistical significance were calculated using GraphPad Prism version 10.4, with unpaired t-tests or one-way ANOVA as appropriate. Results: Bivalent peptide BVE7-001 shows no significant difference with BE1-7(1.23±0.05μM and 0.95±0.02μM, n=4; t-test, $P<0.05$), both show significantly lower potency compared with fentanyl (9.92±1.53nM, n=4; t-test, $P<0.05$). The IC_{50} of BVE7-003 and BVE7-005, bivalent constructs with longer linkers (15.89±1.26nM and 12.42±1.17nM, n=4; t-test, $P<0.05$), show no significant difference with fentanyl. Conclusion: The findings provide evidence that BVE7-003 and BVE7-005 improve the potency of the parental monomeric BE1-7 via bivalent peptide design and potency is consistent with that of the clinically utilised agonist fentanyl. Our studies show a similar benefit with biphalin, as reported by Lipkowski, A. W et al. (1982), a bivalent peptide comprising a modified enkephalin as the pharmacophore, which is shown to be a potent agonist. We propose that bivalent ligands connected by a linker of defined length are considered to enhance local drug concentration and act synergistically to improve the likelihood of targeting specific receptors. Interestingly, BVE7-001 showed no enhancement, which is supposed to result from its short linker. These findings reveal that the linker's length influences the ligand peptide's performance, while proving that the bivalent strategy can improve opioid potency.

Disclosures: Y. Wang: None. **D. Saifuddin:** None. **K. Tupally:** None. **H. Parekh:** None. **P.J. Cabot:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.04/LL9

Topic: H.08. Drugs of Abuse and Addiction

Support: The University of Queensland Graduate School Scholarship Research Training Program

Title: Bivalent Kappa-selective opioid peptides elicit antinociceptive effects on a chronic inflammatory pain model

Authors: *D. A. P. SAIFUDDIN[1], R.-J. LOHMAN[1], K. TUPALLY[2], H. S. PAREKH[3], P. J. CABOT[1];
[1]Sch. of Pharm. and Pharmaceuctical Sci., [3]Sch. of Pharm. and Pharmaceut. Sci., [2]The Univ. of Queensland, Woolloongabba, Australia

Abstract: The opioid pandemic is an urgent global clinical problem. People suffering from chronic pain who are prescribed conventional opioid analgesics (e.g. morphine) suffer from debilitating complications such as addiction. Opioid receptors (OPs) are classified into 4 OP subtypes: Mu (MOP), Delta (DOP), Kappa (KOP) and Nociceptin (NOP). OPs modulate the nociceptive pathway in the central nervous system (CNS) and peripheral nervous system (PNS). MOPs in the CNS are the target for most of the conventional opioid analgesics and are a major contributor to addiction. PNS-acting opioids elicit analgesia, avoiding the CNS. Dynorphin is an endogenous KOP-selective opioid peptide produced by the nervous system and immune cells. Two bivalent constructs (BCs) of Dynorphin 1-7 with varying linker lengths and chemistries were designed, targeting the peripheral KOP. Through *in vitro* assessment, two optimal BCs, DS1 and DS2, were selected for *in vivo* efficacy screening in a chronic inflammatory pain model. All studies were approved by the University of Queensland's Animal Ethics Committee. The Freund's complete adjuvant (FCA) model of hyperalgesia was used in Male Wistar rats. The right paw received FCA, whereas the left was used as an internal control. The anti-nociceptive effects of the compounds were assessed up to 24 hours using the Randall-Selitto method, as measured by paw withdrawal threshold (PWT). Oedema was measured using a plethysmometer by monitoring changes in paw volume. Rats were administered DS1, DS2, and U50,488H (KOP positive control) or saline vehicle in the paw pad in a blinded manner. A KOP antagonist, norbinaltorphimine (NorBNI), was used to determine agonist specificity at KOP. The area under the curve (AUC) was used to determine the efficacy of compounds. Data were expressed as AUC% change against saline ±SEM (n=3-6), statistical significance was determined through ANOVA using GraphPad Prism software, version 10.4.1. FCA treatment caused significant oedema, resulting in a volume change of 166.5±17.3%. Both BCs showed an increase in PWT over time after 30-60 min at 5 mg/kg. DS2 and DS1 achieved 171.25±9.5% and 141.4±10.4%, respectively ($p < 0.005$). Upon administration of NorBNI, neither BC elicited an antinociceptive response, as reflected in insignificant change of 0.07±5.05% and 15.7±14.2% for DS2 and DS1,

respectively. In the absence of NorBNI, DS1 and DS2 elicited an AUC change of 158.9±19.3% and 136.9±17.3% ($p < 0.005$), indicating a prolonged anti-nociceptive effect. The novel BCs show promise as new KOP-selective PNS-acting analgesics. Future work will focus on efficacy in other pain models, pharmacokinetic properties, and side effects profile.

Disclosures: D.A.P. Saifuddin: None. **R. Lohman:** None. **K. Tupally:** None. **H.S. Parekh:** None. **P.J. Cabot:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.05/LL10

Topic: H.08. Drugs of Abuse and Addiction

Support: The University of Queensland-Australia Government Research Training program

Title: Targeting peripheral mu and delta opioid receptors with bivalent opioid peptides for pain treatment

Authors: *J. ZHANG, D. SAIFUDDIN, M. ROBITAILLE, B. ROSS, P. J. CABOT;
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Abstract: Opioids are effective for pain relief by modulating opioid receptors (OPs), which are G-protein coupled receptors (GPCRs), including the mu (MOP) and delta (DOP) subtypes. Opioid addiction and misuse are prevalent issues leading to a significant public health crisis known as the global opioid pandemic. Endogenous opioid peptides (EOPs) have lower side effects and potential efficacy as clinical opioids. β-endorphin (BE) and enkephalin (ENK) are EOPs targeting MOP and DOP. Activation of opioid receptors by agonists elicits downstream regulatory effects mediated by secondary messengers, notably cyclic adenosine monophosphate (cAMP). Bivalent opioids are composed of BE1-7 and Leu-ENK, connected via a linker of varying length such as BBL001, BBL003, and BBL005. They are designed to provide potent analgesia and be peripherally active, potentially reducing opioid-related side effects. Bivalent opioid peptides were synthesised by solid-phase peptide synthesis. The Revvity AlphaScreen© cAMP detection kit was used to quantify cAMP released by HEK293 cells stably expressing MOP, DOP, or MOP/DOP upon stimulation with forskolin. Fentanyl and SNC80 were used as positive controls for MOP and DOP, respectively. A concentration curve of the agonist was used to determine IC_{50}. All experiments were carried out in biological quadruplicate, and statistical significance was assessed via GraphPad Prism version 10.4 using unpaired *t*-tests or one-way ANOVA as appropriate based on the experimental group. BBL003 exhibited similar performance in HEK MOP and HEK DOP cell lines from the cAMP inhibition curve compared with the positive controls, fentanyl and SNC80 (29.5±8.7 nM vs 28.5±9.8 nM (fentanyl), and 19.6±9.9 nM vs 44.4±6.7 nM (SNC80), n=4, $p>0.05$, unpaired *t* test). In the HEK MOP/DOP

cell line, the bivalent peptides BBL003 and BBL005 show stronger efficacy with increased linker length, about 1.5-fold that of the control ($p<0.05$, unpaired t test). Bivalent peptides showed improved efficacy and similar potency compared to the control agonist, suggesting an improvement in OP modulation, especially in the HEK MOP/DOP cell line. The increase in efficacy suggests a dual receptor interaction, which requires further assessment with known dual agonists. BBL003 and BBL005 showed optimal efficacy and potency compared to other peptides and were selected for further studies.

Disclosures: J. Zhang: None. **D. Saifuddin:** None. **M. Robitaille:** None. **B. Ross:** None. **P.J. Cabot:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.06/LL11

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Y1AA-3009

Title: Disturbed autonomic-central coupling in opioid use disorder

Authors: *E. SHOKRI KOJORI, P. MANZA, S. B. DEMIRAL, N. D. VOLKOW;
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Abstract: Opioid use disorder (OUD) is a leading cause of mortality in younger adults in the US. Understanding how OUD affects brain physiology is key for development of effective treatments. There is a growing recognition that the interactions between autonomic and central nervous systems contribute to the spontaneous patterns of brain activity that may support brain health. OUD affects both central and autonomic nervous systems, yet central-autonomic interactions in OUD are poorly understood. Here we studied 37 healthy individuals (13 females, 22-64 years old) and 38 age- and sex-matched individuals with OUD (15 females, 20-64 years old). We performed resting-state fMRI (7.7 min) to measure brain activity and concurrently recorded respiratory and pulse signals to characterize autonomic function. We found OUD relative to healthy individuals had significantly higher fractional amplitude of low frequency (LF: 0.01-0.1 Hz) fluctuations (fALFF) bilaterally in a cluster of medial white matter, parahippocampus, and lentiform nucleus ($p_{FWE} < 0.05$). Group differences in the LF power of the respiratory ($p = 0.7$) and pulse ($p = 0.1$) signals were not significant. Yet, fALFF in the white-matter cluster was associated with LF power of the respiratory signal in healthy individuals ($r(23) = 0.43$, $p = 0.03$) and in OUD ($r(29) = 0.48$, $p = 0.006$). We studied the temporal coupling (indexed by phase coherence) between LF variations in the respiratory/pulse signals and brain fMRI. Significant phase coherence with LF respiratory signal was observed in thalamus, brainstem, and cerebellum, which was not significantly different between groups. Significant phase coherence with LF pulse signal (related to sympathetic function) was observed bilaterally

in autonomic network regions including sensorimotor cortex, calcarine, and thalamus. This effect was lateralized in OUD which showed significantly diminished phase coherence in the left hemisphere ($p_{FWE} < 0.05$, healthy individuals: -0.395 rad vs. OUD: 0.012 rad). Associations between white matter fALFF and LF power of the respiratory signal in both groups are consistent with our recent findings showing slow breathing is associated with increased fALFF in the white matter. Higher white matter fALFF in OUD may indicate increased sensitivity of implicated processes (likely non-neuronal) to respiration. Finally, we provide evidence that individuals with a history of OUD show laterally diminished LF phase coherence with a marker of sympathetic activity that may be related to altered vascular signaling in OUD. Alterations in brain activity and its interactions with autonomic function offer novel perspectives for studying OUD symptomatology.

Disclosures: E. Shokri Kojori: None. **P. Manza:** None. **S.B. Demiral:** None. **N.D. Volkow:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR410.07/LL12

Topic: H.08. Drugs of Abuse and Addiction

Support: R01DA056602
5 T32 GM 133351-3

Title: Behavioral and amygdalar circuit alterations in a mouse model of fentanyl dependence and withdrawal

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Abstract: Fentanyl dependence remains a critical and escalating public health crisis, accounting for over 80,000 overdose deaths annually in the United States. However, the behavioral and neural consequences of fentanyl dependence and withdrawal remain poorly understood. To address this gap, we developed and validated a 4-day escalating-dose model of fentanyl dependence in C57BL/6J mice. This model produces robust somatic and affective withdrawal symptoms and enables investigation into how dependence alters reward processing, aversion sensitivity, and feeding behavior.
To first evaluate core symptoms of withdrawal, mice were treated with fentanyl (2×/day, escalating over 4 days) or saline. Naloxone-precipitated withdrawal elicited significant somatic symptoms (e.g., jumping, hyperalgesia) and was associated with conditioned place aversion (CPA), confirming both physical and affective dimensions of dependence. To assess how withdrawal influences valence processing, we trained food-restricted mice on a two-tone

discrimination task pairing auditory cues with sucrose or footshock. Fentanyl-dependent mice demonstrated heightened sucrose-directed responding and altered responses to punishment cues during spontaneous withdrawal. Conversely, when repeated under free-feeding conditions, sucrose-directed responding appeared abolished, and work is ongoing to quantify fear-associated responses.
To further determine whether increased sucrose-directed responding reflects enhanced reward-seeking or increased hunger, we used home-cage FED3 devices to continuously monitor food intake across treatment and withdrawal. Preliminary data suggest fentanyl-dependent mice reduce intake during treatment without rebound during withdrawal.
To examine how dependence may disrupt valence encoding at the circuit level, we are quantifying cFos expression in projection-defined BLA→CeA and BLA→NAc neurons following naloxone-precipitated withdrawal. To complement this endpoint analysis with real-time activity, we are also recording calcium dynamics from BLA→CeA neurons during CPA expression using in vivo fiber photometry. These ongoing experiments aim to clarify how valence processing is altered across withdrawal states.
Together, this combination of experimental approaches with our fentanyl dependence model enables a multi-level analysis of behavioral and circuit-level adaptations, and may help identify neural substrates underlying withdrawal and relapse vulnerability.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA grant R21DA056177
NIDA U01DA051972

Title: Characterization of regulatory genetic variants that are associated with opioid use disorder

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Abstract: Background: The opioid epidemic has been a primary driver of the overdose crisis in the US. Substance use disorders (SUD) have a strong genetic component, with heritability rates of 40-60%. Genome-wide association studies (GWAS) have identified hundreds of genetic variants associated with drugs of abuse such as opioids. However, the functional impact of many noncoding variants remains uncharacterized.
Methods: I compiled a library of SNPs associated with opioid use disorder (OUD) from well-

powered GWASs. After pulling SNPs in linkage disequilibrium (LD) with lead variants, I fine-mapped and prioritized those affecting regulation in brain tissue by assessing possible target genes and overlap with accessible chromatin, transcription factor binding sites, and known eQTLs. This library will be tested in induced pluripotent stem cell-derived neurons, using the transcription start site massively parallel reporter assay (TSS-MPRA). TSS-MPRA profiles the 5' RNA ends of barcoded reporter constructs at single-nucleotide resolution, providing a functional readout of transcriptional activity at putative regulatory elements. To test a mouse model of fentanyl dependence, our lab tested a 4 day escalating dose schedule to induce differential RNA expression. n=5 male mice were used for each treatment group. The fentanyl group received an escalating dose of fentanyl (0.7 mg/kg, 0.9 mg/kg, 1.3 mg/kg, 1.3 mg/kg) twice a day starting on Day 1. The saline group received saline injections twice a day at similar times. On day 5, no fentanyl or saline was administered and mice were allowed to go into spontaneous withdrawal for the next 4 days. Amygdala brain punches were collected and processed for bulk RNA-seq and analyzed with R.
Results: Of the library, around 12% of the SNPs overlapped with open chromatin regions in neural cell types, 27% of them directly overlapped a transcription factor motif, and 11% of them were known eQTL sites. Overall, 188 SNPs showed potential for a regulatory role in the brain, indicating relevance to fentanyl response. The RNA-seq results showed over 1200 differentially expressed genes. Among these, the target genes of many transcription factors, such as Tcf4, Mef2c, and Sox2, were enriched. These results help contextualize important regulatory pathways that may be affected by SNPs overlapping enriched transcription factor motifs.
Conclusion: The results from these experiments will help evaluate my central hypothesis that the modulation of cis-regulatory elements via inherited SNPs plays a role in the response to opioid exposure and the development of dependence.

Disclosures: M.A. Williams: None. **S. Desfor:** None. **L. Liaw:** None. **A. Kumar:** None. **F. Telese:** None.

Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.09/LL14

Topic: H.08. Drugs of Abuse and Addiction

Title: Characterizing molecular regulators of striato-cortical ensembles regulating intravenous fentanyl intake and seeking

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[1]Psychiatry, Univ. of California San Diego, San Diego, CA; [2]Psychiatry, UCSD, La jolla, CA; [3]Psychiatry, UCSD, La Jolla, CA

Abstract: Opioid Use Disorder (OUD) affected 16 million people globally as of 2024. OUD is a condition marked by recurrent drug intake and seeking behavior. These behaviors are encoded by

Fos-expressing neuronal ensembles. Ensembles are defined as small, sparsely distributed populations of neurons selectively activated and deactivated in striatal-cortical brain regions to facilitate long-term opioid intake. Reln, a gene encoding the extracellular matrix protein Reelin, may have a suggested function as a molecular gatekeeper of cocaine-induced ensembles. However, it remains unknown whether Reln also regulates ensemble formation in response to synthetic opioids, such as fentanyl. To address this, we developed an intravenous self-administration (IVSA) paradigm in young adult mice featuring 6 phases: acquisition, dose-response, restabilization, progressive ratio, extinction and cue induced reinstatement. Using this paradigm, we tested the functional role of Reln on fentanyl-driven behavior by comparing lever presses of the heterozygous Reeler mice (Het) versus wild-type (wt). There were no detectable significant differences in drug taking between the Het versus wt mice during IVSA sessions. During all IVSA phases, both genotypes displayed increased interaction with the auditory and visually cue-paired lever reinforced with fentanyl infusions compared to a non-reinforced lever. During the extinction phase, both genotypes showed a loss of lever discrimination. Despite lack of changes in the IVSA and extinction phase, cue-induced reinstatement led wt mice to display a stronger drug seeking phenotype compared to the Het mice. These results suggest that Reln regulates re-activation of fentanyl seeking ensembles. To better understand the role of Reln in regulating fentanyl-seeking ensemble dynamics, we are examining Fos expression in wt and Het mice 30 minutes after cue-induced reinstatement session. Fos-expressing ensemble quantification is ongoing in the dorsomedial striatum and prefrontal cortex, where we observed pronounced Fos expression after acute fentanyl exposure. To further dissect the role of Reelin in fentanyl-linked behavior, current experiments are manipulating Reln expression in an ensemble- and tissue-specific manner. Findings from this study could elucidate the potential role of Reln in the formation and plasticity of ensembles underlying fentanyl dependence.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA P01DA047233
NIDA R01DA014133

Title: Long-term fentanyl withdrawal reprograms nucleus accumbens gene networks and reward-related behavior

Authors: *E. S. KAHN[1], B. T. KIPP[2], V. KONDEV[2], T. M. GYLES[6], M. ESTILL[2], E. P. CHEN[3], G. ROJAS[4], Y. YIM[5], E. J. NESTLER[7];
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Friedman Brain Inst., [5]Nash Family Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [6]Friedman Brain Inst., [7]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Fentanyl, a potent synthetic opioid, is a major contributor to the current overdose epidemic, yet its long-term molecular and behavioral effects remain incompletely understood. Using bulk RNA sequencing of the nucleus accumbens (NAc) in male and female mice following chronic exposure and withdrawal, we observed largely distinct transcriptional responses to fentanyl and morphine at 24 hours post-exposure at doses that elicit similar behavioral effects. However, after 30 days of withdrawal, transcriptional profiles converged dramatically, suggesting shared long-term neuroadaptive processes. Gene co-expression network analysis identified a module (c1.7), enriched for genes involved in synaptic plasticity, that is robustly upregulated in the NAc during prolonged withdrawal from both opioids. To further explore the regulatory basis of these persistent transcriptional changes, we are integrating RNA-seq with chromatin accessibility profiling from assay for transposase-accessible chromatin sequencing (ATAC-seq). In parallel, we characterized behavioral consequences of fentanyl exposure and withdrawal using the platform mediated avoidance (PMA) task and a battery of assays designed to assess core addiction-related behaviors, including reward sensitivity, risk-taking, and decision-making under conflict. We show that fentanyl exposure alters reward-seeking and risk-taking behaviors observed across multiple paradigms, suggesting persistent disruptions in natural reward processing and threat valuation that may underlie relapse vulnerability. Together, our findings reveal parallel molecular and behavioral adaptations to fentanyl withdrawal and suggest potential epigenetic mechanisms regulating long-term plasticity in opioid addiction.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

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Arizona Department of Health Services (ADHS) and the state of Arizona (ADHS Grant No. CTR057001)

Title: Effects of repeated oxycodone in mice: Changes in behavior and oligodendrocyte-related gene expression

Authors: *O. LAW[1], J. C. GEWIRTZ[2], J. L. VERPEUT[3];
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Abstract: Opioid Use Disorder (OUD) constitutes an epidemic that imposes a substantial burden on health and economic wellbeing in the United States and worldwide. A key set of symptoms of OUD is the experience of negative affect. Recent research suggests the involvement of myelination-related gene activity in the sequelae of opioid administration, including negative affect (Liu et al., 2025; Yalcin et al., 2025). The aim of the current study was to examine the acute and withdrawal effects of oxycodone (5, 10, 20 or 30mg/kg) following repeated administration of oxycodone (30mg/kg/day for 10 days) in C57Bl/6J mice (n = 21). We hypothesized that withdrawal would reduce sociability, increase anxiety, and alter expression of genes related to the control of oligodendrocyte cell lineage. The acute effects of oxycodone were measured using a hot plate and an open field. Thermal nociception was quantified at 15, 30, and 45 minutes post-injection of a single oxycodone dose (5, 10, 20 or 30mg/kg) or saline. The 30mg dose of oxycodone was the only dose to show higher analgesia at all three timepoints ($p < 0.01$). Machine learning analysis in the open field showed that oxycodone (30 mg/kg) injected mice increased their distance traveled ($p = 0.002$) relative to controls. These findings confirm the functional efficacy of the highest dose of oxycodone. Next, we analyzed the impact of withdrawal on social behavior and anxiety. In the open field, social interactions of 3 cagemates were recorded at baseline and during 24hr withdrawal. Oxycodone-withdrawn animals demonstrated decreased counts of social behavior compared to baseline ($p < 0.001$) but showed no differences in total duration of social behavior. Finally, in the marble burying task, 48hr oxycodone-withdrawn animals buried fewer marbles compared to controls ($p = 0.04$). Following marble burying, the prefrontal cortex (PFC) was extracted for RT-qPCR to analyze expression of five genes functionally implicated in multiple stages of oligodendrocyte maturation: *Tc7fl2*, *Plp1*, *Olig1*, *Klk6*, and *Mbp*. Of these, *Tcf7l2* ($p = 0.009$) and *Klk6* ($p = 0.045$) showed decreased expression in oxycodone animals relative to controls. Both genes are involved in the early stages of oligodendrocyte differentiation. In conclusion, the acute administration of 30-mg/kg oxycodone resulted in increased thermal analgesia and increased locomotion. Withdrawal reduced marble burying, social behavior in the open field, and expression of *Tcf712* and *Klk6*. Thus, this study provides evidence of dysregulated myelination-related gene activity in the PFC during oxycodone withdrawal, supporting the potential utility of treatments that target oligodendrocyte function.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR410.12/LL17

Topic: H.08. Drugs of Abuse and Addiction

Support: DA000069
NIH / NIDA IRP

Title: Fluornitazene metabolite DFNZ: A highly selective mu opioid receptor superagonist with potent and protracted analgesic qualities

Authors: *J. L. GOMEZ[1], Z. J. FRANGOS[1], A. E. TISCHER[1], A. SULIMA[1], J. M. BOSSERT[1], L. SHI[1], R. HUTTENHAIN[2], J. BONAVENTURA[3], Y. SHAHAM[1], V. ZACHARIOU[4], K. C. RICE[1], M. MICHAELIDES[1];
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Abstract: Nitazenes, an obscure class of synthetic benzimidazole opioids, are increasingly being identified in the recreational drug supply. Originally developed for pain treatment, their use was discontinued due to safety concerns. Our research has focused on desethyl-FNZ (DFNZ), the major metabolite of fluornitazene (FNZ), and we have characterized its properties, building upon our previous work with FNZ. While DFNZ exhibits analgesic and behavioral qualities similar to FNZ, its pharmacology is distinctly unique. Despite differing by only a single ethyl group chemically, these two compounds show a clear divergence in functional efficacy and potency. New data from dynamic BRET assays and proteomics further clarify the efficacy profile of DFNZ and FNZ, revealing distinct engagement with downstream signaling pathways that likely contribute to their observed pharmacological properties. Furthermore, DFNZ has demonstrated efficacy in chronic pain models, suggesting its potential for long-term pain management. Importantly, DFNZ has shown promising results in antagonizing heroin seeking in preclinical models, indicating a potential for reduced abuse liability. This is supported by self-administration experiments that show weak conditioned reinforcement for DFNZ and lower motivation to infuse the drug. Dopamine sensor data corroborates these findings: while FNZ, morphine, and fentanyl all increase dopamine signals following intraperitoneal injection, DFNZ does not. Another crucial aspect of abuse liability is the severity of withdrawal following chronic drug use. Following chronic administration, DFNZ did not produce meaningful withdrawal symptoms, which may also contribute to the weak reinforcement characteristics observed. In conclusion, DFNZ demonstrates an unexpectedly strong safety profile for a nitazene MOR superagonist. These findings have important implications for both pain and addiction treatment, challenging the conventional notion that high-efficacy MOR agonists cannot constitute safe and effective therapeutics.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.13/LL18

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA-IRP

Title: A novel negative reinforcement paradigm to examine the brain regions involved in withdrawal avoidance

Authors: *C. TRUMAN-WYSS[1], H. JEDEMA[3], J. L. GOMEZ[2], M. MICHAELIDES[2], A. JOHANSEN[4];
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Abstract: Opioid use is driven by both positive reinforcement (experiencing rewarding effects) and negative reinforcement (avoiding withdrawal). While opioid research has predominantly focused on positive reinforcement, negative reinforcement plays a critical role in opioid use and relapse. Preclinical studies showed increased opioid self-administration during withdrawal, highlighting the importance of negative reinforcement in opioid addiction. Moreover, brain regions including the anterior cingulate cortex (ACC), central amygdala, and ventral forebrain show elevated activity during withdrawal states. However, the neural circuits underlying active withdrawal avoidance remain poorly understood. To address this gap, we developed a novel negative reinforcement task in rhesus macaques (N=3). Subjects were trained to initially escape an aversive air puff by pressing a symbol on a touchscreen after air puff initiation. Subsequently, they learned to avoid the air puff by pressing the symbol within 2 seconds of its appearance prior to air puff initiation. Both escape and avoidance tasks were interleaved with water reward trials, with a ratio of 80:20 reward to escape/avoidance trials. All subjects quickly learned to escape (~96% ± 2.5 successful response rate) and avoid the air puff stimulus (~96% ± 2.8 successful response rate) within approximately two weeks of training. Moreover, subjects displayed higher attentiveness during negative reinforcement trials compared to positive reinforcement trials, with an average successful positive reinforcement trial response rate of 75% (± 3.5). Next, we will establish morphine dependence and subjects will learn to escape/avoid naloxone infusions administered intravenously via a vascular access port. Finally, subjects will perform a joint task, consisting of alternating blocks of air puff and naloxone avoidance trials. Sessions will occur in a positron emission tomography (PET) scanner, which measures dynamic, task-dependent changes in metabolism in a single session using the radiotracer [^{18}F] fluorodeoxyglucose (FDG). While monkeys are awake and undergoing the task, neural activation will be measured via a function PET (fPET) technique and compared between air puff and naloxone avoidance blocks. Our work aims to elucidate the neural substrates involved with opioid withdrawal avoidance, identifying novel targets for intervention. Research was supported by NIDA-IRP.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

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Program #/Poster #: PSTR410.14/LL19

Topic: H.08. Drugs of Abuse and Addiction

Title: The effects of n-desethyl-fluornitrazene on remifentanil choice in squirrel monkeys

Authors: *A. J. MYERS[1], M. ZARCONE[3], J. L. GOMEZ[4], M. MICHAELIDES[2], H. P. JEDEMA[5];
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Abstract: Opioids as a class of drug are highly effective analgesics but come with significant adverse effects such as respiratory depression, tolerance, dependence, withdrawal, and addiction. The development of new μ opioid receptor (MOR) agonists that are effective and safe could have wide-ranging implications for effective pain management amid the opioid epidemic. N-desethyl-fluornitrazene (DFNZ) is a MOR superagonist derived from a class of synthetic opioids called nitazenes that has shown a promising combination of effective analgesia and minimal adverse side effects, including a lack of induced respiratory depression, tolerance, and only weak withdrawal induction, in rodents. In addition, DFNZ has been shown to reduce heroin self-administration. Taken together, DFNZ appears to have potential as a pain management tool and a novel opioid maintenance treatment, warranting further study. In the present study we aim to examine the effects of this novel MOR superagonist on economic choice between remifentanil and a food reward in squirrel monkeys. During daily sessions, squirrel monkeys ($n=7$) chose between stimuli associated with different quantities of intravenous infusion of remifentanil (0.08-0.32μg/kg) and sweetened condensed milk (0.045-0.6ml/kg) in a well below the touchscreen. Choice allocation between remifentanil and milk during a week of DFNZ pretreatment (10 minutes prior) was compared to baseline choice allocation the week prior. The primary outcome of interest is the indifference value, or the drug to milk ratio where subjects display an equal probability of selecting drug or milk. An initial low dose of DFNZ (30 μg/kg) caused a slight reduction in remifentanil choice and an associated increase in indifference values. We are currently exploring the effects of additional doses. Research was supported by NIDA-IRP

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

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Topic: H.08. Drugs of Abuse and Addiction

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Title: Habenular μ-opioid ensembles contribute to opioid aversion and protracted withdrawal states

Authors: *J. A. G. SOARES[1], S. N. SUTLEY-KOURY[2], M. ASIM[2,3], M. B. POMRENZE[2,3], R. C. MALENKA[2,3], J. TUCCIARONE[2];
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Abstract: Repeated opioid exposure recruits aversion-related brain circuits, altering reward processing and promoting maladaptive states that persist during abstinence. Understanding the specific neural substrates vulnerable to opioids is critical for developing targeted therapies for dependence and relapse. The μ-opioid receptor (MOR), a primary target of opioids, is densely expressed in the habenula (Hb)—a key aversion center comprising medial (MHb) and lateral (LHb) subdivisions. Here, we hypothesized that MOR-expressing Hb neurons contribute to opioid aversion learning and behavioral impairments associated with protracted withdrawal. Using gene knockout and targeted recombination in active populations (TRAP), we investigated the role of MOR-expressing Hb ensembles in: (1) the expression of aversive learning and

physical symptoms during naloxone-precipitated withdrawal, and (2) the emergence of behavioral deficits following prolonged abstinence. TRAP2xAi14 mice administered 4-hydroxytamoxifen (4-OHT) immediately after naloxone (5 mg/kg, i.p.) displayed enhanced recruitment of neurons in both MHb and LHb. Selective MOR deletion in the LHb attenuated naloxone-induced conditioned place aversion without affecting the somatic signs of acute withdrawal. Mapping of c-fos neuronal activity during both precipitated and protracted withdrawal revealed substantial overlap in the active neuronal populations across these states. Chronic fentanyl exposure induced social deficit, despair-like behavior, and hyperalgesia three weeks into abstinence. Targeted ablation of MORs in the Hb of MORfl/fl mice prevented social deficits while exacerbating hyperalgesia during protracted withdrawal. Together, these findings support a critical role for Hb MOR neurons in mediating aversive learning and in driving long-lasting behavioral dysfunctions following opioid exposure. Ongoing work in our lab employs chemogenetics and fiber photometry to further dissect maladaptations in this circuitry and their contribution to withdrawal-related behavioral states. Additionally, we are characterizing MOR-expressing Hb populations and their projections using RNAscope and neuroanatomical mapping. This more elaborate circuit dissection could have important implications in negative reinforcement and states of physical and emotional suffering that drive opioid relapse.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR410.16/LL21

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH/NIDA IRP DA000069

Title: Investigating the therapeutic potential of ketamine to rescue neurobehavioral effects of withdrawal in morphine-dependent rats

Authors: *A. E. TISCHER[1], Z. J. FRANGOS[1], G. TANDA[2], M. MICHAELIDES[1]; [1]Neuroimaging Res. Br., [2]Medication Develop. Program, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Relapse in opioid use disorder (OUD) can be driven by the aversive physical and affective symptoms of withdrawal. Rodent models of naloxone-precipitated opioid withdrawal have identified changes in dopaminergic neurotransmission and endogenous opioid peptide function that may contribute to this aversive state. Rescuing these signaling changes pharmacologically may alleviate withdrawal-associated behaviors. Ketamine has recently emerged with clinical potential to alleviate buprenorphine-precipitated withdrawal; however, its mechanism is unclear. Here, we established a model of morphine dependency and naloxone-precipitated withdrawal with the intention of assessing the efficacy of pharmacotherapies to alleviate withdrawal signs. Male and female Sprague Dawley rats were continuously exposed to escalating morphine doses (1.6-25.6 mg/kg from Day 1-10) via subcutaneous osmotic minipumps. On Day 10, naloxone (1 mg/kg, i.p.) or saline (control) was administered to precipitate withdrawal. Somatic withdrawal signs were assessed, and [^{18}F]FDG PET was used to examine changes in brain metabolic activity. In a separate cohort, we used autoradiography to evaluate mu opioid receptor (MOR) occupancy and density with [^{3}H]DAMGO and DAMGO-induced G protein activation with [^{35}S]GTPγS. Naloxone pre-treatment produced significant somatic withdrawal signs and reduced locomotion in the morphine-dependent rats. PET scans showed decreased activity in the somatosensory cortex and increased activity in the nucleus accumbens, septum, hypothalamus, amygdala, and thalamus of morphine-dependent rats pre-treated with naloxone compared to saline. We observed ~20% occupancy of MOR by morphine in the caudate putamen and no significant changes in MOR density or G protein activation between saline- and morphine-treated groups. Overall, we developed a robust model of morphine dependency and identified brain regions differentially activated during withdrawal. Next, this model will be used to test the efficacy of ketamine and its enantiomers to alleviate withdrawal signs. We will use concurrent microdialysis and fiber photometry to explore whether ketamine alleviates withdrawal-related behaviors by restoring dopamine and endogenous opioid signaling in PET-identified brain regions.

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Poster

PSTR410: Opioid Addiction, Dependence, and Pain: From Genes to Behavior

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Program #/Poster #: PSTR410.17/LL22

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant 5U01DA051947-04

Title: Neuronal subtypes, transcriptional signatures, and chromatin accessibility states of the ventral pallidum during forced abstinence to heroin

Authors: *B. GRISSOM[1], M. GREEN[2], R. R. CAMPBELL[3], S. MITRA[4], S. A. AMENT[5], D. M. DIETZ[6], M. LOBO[7];

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Abstract: Opioid addiction is characterized by the persistence of drug-craving even after long periods of abstinence, leading to recurring cycles of drug-seeking and relapse. The ventral pallidum (VP) may play an important role in these processes as a central node of both the mesolimbic reward system and of extended amygdala circuits activated during withdrawal. However, the cell type diversity of the VP and the molecular adaptations associated with substance use and addiction are poorly characterized. To address this, we sequenced the nuclear transcriptomes (snRNAseq) of 92,978 cells and chromatin accessibility states (snATAC-seq) of 47,050 cells from the VP of rats in the context of heroin self-administration and forced abstinence at time-points 1 and 14 days post final self-administration session. We generated a multimodal atlas for the diversity of neuronal and non-neuronal cells including 48 transcriptionally distinct neuronal subtypes in the VP and surrounding regions, as well as a continuous spectrum of maturing oligodendrocyte precursor cells. We verified the spatial localizations of these cell types via subcellular-resolution spatial transcriptomics using the CosMx platform. Heroin self-administration and forced abstinence were associated with gene expression changes suggesting neuronal subtype-specific effects on epigenetic regulation and neuroplasticity. We further confirmed differential expression of genes in glutamatergic VP neuron populations using RNAscope. We integrated our snRNA-seq and snATAC-seq data to model gene regulatory networks mediating these effects. Our results provide insight into the gene regulatory mechanisms mediating both the transient and persistent effects of opioids through the VP. The identification of high-impact high-specificity transcriptional and neuroplastic mechanisms will open promising new avenues for treatment research.

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Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.01/MM1

Topic: I.05. Working Memory

Support: NIH R01 EY-016407
NIH R01 EY-033925
NIH R90 DA060339

Title: Putting visual working memory to work

Authors: *Z. DUAN[1], N. TARDIFF[1], C. E. CURTIS[1,2];
[1]Psychology, [2]Cns, New York Univ., New York City, NY

Abstract: Human neuroimaging studies of visual working memory (WM) have focused on understanding how and where visual memoranda are stored. However, WM has not only storage but also control components. For example, WM contents can be manipulated or 'worked on' in support of goal-directed behaviors, transforming the memoranda from what was originally perceived and stored. Here, we tested the hypothesis that WM contents can be transformed across multiple brain regions in service of task demands. Human participants (N = 15) performed three WM tasks in the fMRI scanner. In each task, two oriented gratings were peripherally presented on each side of the screen, preceded by a pre-cue indicating the task-relevant item. During the WM delay, the baseline task required participants to remember the orientation of the cued item at its original location. The rotate-only task asked participants to mentally rotate the cued item's orientation counterclockwise 45° and then maintain the rotated orientation at its original location. The rotate+remap task asked participants to first rotate the cued item's orientation and then remap it to the other side of the screen. We used a separate retinotopic mapping task to identify each voxel's population receptive field (pRF) and define regions of interest (ROIs), including occipital, parietal, and frontal cortex. To examine neural dynamics of voxels with different pRFs during the spatial remapping process, we defined three subregions within each ROI in screen coordinates relative to the cued item (contralateral, ipsilateral, and foveal) based on the estimated pRF parameters. After stimulus onset, we found that response amplitudes were initially higher in the contralateral subregions and then shifted to the ipsilateral subregions, if spatial remapping was required. Additionally, voxel activities in foveal subregions were first suppressed and then elevated during the latter delay, suggesting that feedback signals target the foveal retinotopic cortex. Next, we employed an inverted encoding model (IEM) to investigate whether regions known to be involved in WM storage differentially contribute to rotation and remapping processes. Across visual field maps, we found that the orientations in WM identified by the IEM were rotated in the two tasks that required mental rotations, while the baseline task contained only the original orientation. Orientation transformations were especially pronounced in the fovea. These results suggest that memory transformations occur in early and mid-level visual areas and may depend on remapping peripheral visual features in WM to a workspace in central vision.

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Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.02/MM2

Topic: I.05. Working Memory

Title: Working memory synchronizes oscillations within and across cortical areas

Authors: *M. DAKE[1], S. DANDEKAR[1], C. E. CURTIS[2];
[1]Dept. of Psychology, [2]Psych & CNS, New York Univ., New York, NY

Abstract: Working memory (WM) enables transient maintenance of information necessary to guide goal-directed behavior and is essential for a wide repertoire of cognitive operations. Here we tested the hypothesis that beta-band oscillatory activity plays a key role in supporting behaviorally-relevant stimulus representations during WM. To investigate this, we recorded whole-brain activity using magnetoencephalography (MEG) while human participants performed a memory-guided saccade (MGS) task. On each trial, participants were briefly presented with a visual target at a specific location, followed by a delay period during which they maintained the target's location in memory. Upon a response cue, participants indicated the remembered location by executing a saccade. We observed that during the WM delay, beta-band oscillatory activity in visual cortex exhibited a) dynamic, topographically specific power changes, b) item-specific encoding of the remembered location, c) increased phase synchronization with prefrontal cortex (PFC), and d) predictive relationships with trial-wise memory performance. These findings suggest that synchronous beta-band activity within and between visual and prefrontal areas plays a role in sustaining visual WM representations. Moreover, they have implications for understanding the mechanisms by which WM representations are supported in a widely distributed network of brain areas.

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Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

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Program #/Poster #: PSTR411.03/MM3

Topic: I.05. Working Memory

Support: NIH R01 EY-016407 (CEC)
R01 EY-033925 (CEC)
NYUAD Research Institute Grant CG012 (KKS)

Title: Does neural activity in human V1 persist during working memory, and if not, why?

Authors: *W. CHU[1,3], S. MASTER[1], T. C. SPRAGUE[4], H.-H. LI[5], K. K. SREENIVASAN[3,1], C. E. CURTIS[1,2];
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Abstract: Despite ample evidence that working memory (WM) content can be decoded from human primary visual cortex (V1), activity measured in single neurons in macaque V1 rarely persists above the pretrial baseline. Given that persistent activity is the most established neural mechanism supporting WM, its absence in V1 has led many to conclude that V1 is not involved

in WM storage. Here, we address this contradiction by carefully estimating delay period activity in human V1 from four highly powered fMRI studies of spatial WM that successfully decoded or reconstructed WM content from V1. Leveraging population receptive field (pRF) mapping, we estimated BOLD responses in V1 voxels whose pRFs contained the memory target location (in-RF) and compared these to voxels that did not (out-RF). Across all four datasets, the amplitude of BOLD activity in the in-RF voxels was greater than in the out-RF voxels during the entire memory retention interval, demonstrating an effect of WM content on V1 neural responses. The temporal evolution of the BOLD time courses were revealing: following a transient increase in BOLD activity time-locked to the visual target onset, the in-RF response returned to pretrial baseline levels and the out-RF response dipped well below baseline. Therefore, human V1 activity during WM delays did not persist in the traditional sense (i.e., remain above pretrial baseline); instead, spatially-selective differences persisted between in-RF and out-RF subpopulations. This pattern was unique even when compared to other visual field maps in early visual cortex, where in-RF responses were memory-specific and persisted above pretrial baseline. Additionally, we asked what specialized mechanisms in V1 might account for this paradox by weighing known biological features of visual cortex and a family of computational mechanisms including lateral inhibition, surround suppression, normalization, recurrent connectivity, and feedback. Together, these findings explain why one can decode WM features from V1 and provide mechanistic insights into why V1 activity does not persist during WM.

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Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

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Program #/Poster #: PSTR411.04/MM4

Topic: I.05. Working Memory

Support: NIH R01 EY-016407
NIH R01 EY-033925

Title: Characterizing neural population dynamics of human working memory

Authors: *Z. LU[1], H.-H. LI[3], C. E. CURTIS[2];
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Abstract: The canonical model of working memory (WM) posits that stable, persistent neural activity supports the maintenance of WM representations. However, recent neurophysiological and human neuroimaging evidence suggests that the format of WM changes dynamically during the delay period. A key barrier to understanding these dynamics in humans has been the lack of appropriate analytical tools to track how WM content changes over time. Here, we applied

dimensionality reduction to decompose multivoxel fMRI signals into low-dimensional subspaces with distinct temporal dynamics during WM delay. We hypothesized that an early perceptual subspace encodes stimulus features, while a later subspace encodes task-relevant mnemonic information. Human participants performed a spatial WM task requiring either maintenance or manipulation of location information. Each trial began with a precue indicating the hemifield of the WM target, followed by two peripheral dots sampled pseudorandomly from locations in the periphery. After an initial delay, an auditory cue instructed participants to either maintain the original location (maintaining) or a location remapped 180° across the midline (remapping). We used Principal Component Analysis (PCA) to define early stimulus and late memory subspaces, and projected delay-period activity into each. In maintaining trials, the projected area (or variance explained) in the low-dimensional subspaces decreased when projecting early activity into the late subspace and vice versa, indicating shifting subspaces over time. In remapping trials, this reduction in projected area was even greater, and trajectories between early and late time points showed sharp transitions near the time of the cue, reflecting a dynamic transformation pulled by the remapped location. Furthermore, we find that the stability of WM codes increases along the visual-to-parietal hierarchy in maintaining trials. In remapping trials, the visual cortex showed a similar increasing stability with cortical hierarchy, but early parietal regions were more dynamic, with increasing stability further up the hierarchy, suggesting separate neural subspaces in the parietal cortex coding for distinct aspects of WM. These findings demonstrate that dimensionality reduction can uncover low-dimensional subspaces of WM content from human fMRI data and reveal how neural representations dynamically adapt to task demands.

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Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

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Program #/Poster #: PSTR411.05/MM5

Topic: I.05. Working Memory

Support: NINDS 5K12NS129164-02
K23 NS114178
NIMH MH122023

Title: Bursting in a frontotemporal circuit supports human working memory

Authors: *V. OMELYUSIK[1], T. DAVIS[3], S. S. NAIR[1], B. NOUDOOST[4], P. HACKETT[2], E. H. SMITH[5], S. RAHIMPOUR[5], J. D. ROLSTON[6], B. KUNDU[2];
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Abstract: Increased high gamma (70-140 Hz) and decreased beta (12-30 Hz) band bursting in non-human primate prefrontal cortex (PFC) has been associated with working memory (WM) processing. However, WM is likely related to activity from a network of frontal executive and posterior sensory areas involved in stimulus perception. Here we tested whether high gamma and beta bursting exists in PFC and multisensory lateral temporal areas in humans during visual WM, and whether these areas are coupled via a phase-burst code. We analyzed intracranial local field potential recordings from 7 epilepsy patients performing a load-3 visual WM task. We focused on two brain regions, the middle frontal gyrus (MFG), which includes human dorsolateral PFC, and the middle temporal gyrus (MTG), an area important for multimodal sensory processing. High gamma bursting increased in left MFG during encoding and maintenance while beta bursting decreased (ps<0.05, cluster-based permutation testing). Conversely, beta bursting in right MTG increased during encoding and remained high during the delay period (ps<0.05, cluster-based permutation testing). This effect varied significantly with the subjects' performance on the task. Network analysis showed increased top-down control of PFC onto multisensory areas on correct trials. Specifically, the delay-period high gamma bursting in right MTG was phase-locked to beta phase in left PFC. We quantified this phase-burst coupling (PBC) as a measure that compares the entropy of the experimental phase-burst distribution with the entropy of the uniform distribution and reflects the degree to which bursts cluster at particular phases of the beta wave. Delay-period high gamma bursting in temporal areas was phase-locked to beta phase locally as well as to beta phase in PFC on correct trials. Our findings suggest that WM is supported by frontotemporal network coupling, and that bursting may be the mechanism by which information is stored until readout.

Disclosures: V. Omelyusik: A. Employment/Salary (full or part-time): Kundu Lab – University of Missouri, Columbia. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS 5K12NS129164-02. **T. Davis:** None. **S.S. Nair:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; MH122023. **B. Noudoost:** None. **P. Hackett:** A. Employment/Salary (full or part-time): Kundu Lab – University of Missouri, Columbia. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS 5K12NS129164-02. **E.H. Smith:** None. **S. Rahimpour:** None. **J.D. Rolston:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS K23 (NS114178). **B. Kundu:** A. Employment/Salary (full or part-time): Kundu Lab – University of Missouri, Columbia. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS 5K12NS129164-02, NREF NSRG-P5164_20180321_170626_FE.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.06/MM6

Topic: I.05. Working Memory

Support: NINDS 5K12NS129164-02
K23 NS114178
NIMH MH122023

Title: Item-specific spectral power representations replay during working memory

Authors: *D. R. FAGUE[1], T. DAVIS[2], E. H. SMITH[3], P. HACKETT[5], V. OMELYUSIK[6], B. NOUDOOST[4], J. D. ROLSTON[9], S. S. NAIR[7], B. KUNDU[8];
[1]Electrical and Computer Engin., Univ. of Missouri, Columbia, Columbia, MO; [3]Neurosurg., [4]Ophthalmology and Visual Sci., [2]Univ. of Utah, Salt Lake City, UT; [6]Electrical Engin. and Computer Sci., [5]Univ. of Missouri, Columbia, MO; [7]Univ. of Missouri, Columbia, MO, ; [8]Neurosurg., Univ. of Missouri, Columbia, MO; [9]Neurosurg., Brigham and Women's Hosp., Newton, MA

Abstract: Working memory (WM) involves the short-term maintenance of information online. The sensory WM hypothesis suggests that memory representations are kept online in sensory areas during the memory period. Other data suggests hippocampus, which participates in long-term memory, may also play a role in WM. Representational similarity analysis allows for quantification of how much a multivariable representation derived at one time point is replayed at another time point of a task. We collected data from 7 human subjects with intracranial electrodes implanted for seizure monitoring. The subjects performed a load 3 visual delayed-recognition task with a delay period of 4000 ms. Subjects first fixated on a marker in the middle of the screen for 1000 ms, then were presented 3 images, encoding each image one at a time for 500 ms each, then maintained all 3 images through the 4000 ms delay period, and finally recalled them after that period. Trial-by-trial spectral power from 1-40Hz was calculated for each time point in hippocampal and lateral temporal cortex channels. Similarity was calculated between time points of the encoding period and the maintenance period (EMS) or the encoding period and the fixation period (EFS). Significant replay during maintenance was determined based on baseline replay found during a non-memory fixation period using a cluster-based permutation test on the EMS versus EFS (all significant clusters have $ps<0.05$). We found that the item-specific encoding period spectrum replays during the delay period in ~60% of hippocampal and middle temporal gyrus channels across patients. These data suggest that hippocampus as well as multimodal sensory areas such as middle temporal gyrus may be involved with maintaining memory representations online during WM.

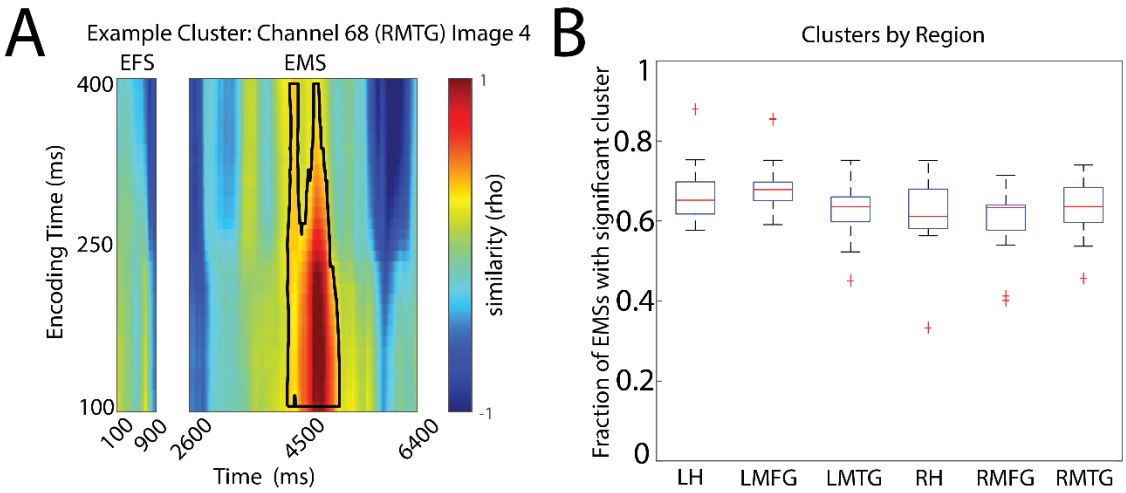

Figure 1. A) Example of an encoding-fixation similarity matrix (EFS) and an encoding-maintenance similarity matrix (EMS) for one trial pair where image 4 was encoded in both trials. Trial X's encoding is correlated with Trial Y's fixation and maintenance. A cluster of the image's encoding being replayed has been identified in the EMS (black boundary). **B)** Distribution of clusters found across all patients, images, and channels within left hippocampus (LH), left middle frontal gyrus (LMFG), left middle temporal gyrus (LMTG), right hippocampus (RH), right middle frontal gyrus (RMFG), and right middle temporal gyrus (RMTG).

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Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

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Program #/Poster #: PSTR411.07/MM7

Topic: I.05. Working Memory

Support: ERC StG MEMCIRCUIT 758032

Title: Conserved single-neuronal mechanisms of short-term memory in the mouse and human prefrontal cortex

Authors: *V. HOHENDORF[1,2,4], L. M. HELD[2], R. BRINI[1], R. DEMANN[5], A. WAGNER[3], B. MEYER[3], J. GEMPT[6], S. N. JACOB[2];
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Abstract: Maintenance of sensory information in short-term memory is an important function of the prefrontal cortex (PFC) and the foundation of intelligent behavior. Here, we investigated how differing task demands shape the underlying representations and directly compared mouse and human recordings at single-neuron resolution. We administered a spatial short-term memory task in two variants that differed with respect to behavioral requirements. In the delayed response (DR) task, spatial information (sample locations) could be directly translated to a behavioral choice (motor action), which was executed after a delay. In the working memory (WM) task, spatial information had to be actively maintained in memory, and the correct motor action could only be determined after a subsequently presented test location. Mice showed faster learning and higher performance in DR compared to WM tasks. In expert sessions of both tasks, we microendoscopically imaged medial PFC (mPFC) pyramidal neurons expressing GCaMP6f (1319 neurons from 6 mice). Individual neurons encoded spatial locations and the animals' behavioral choices, exhibiting monotonic and labelled line tuning patterns. Importantly, during presentation of the sample, the fraction of selective units increased earlier and more strongly in the WM task than in the DR task (peaking at 25% and 14%, respectively). More units also remained selective during the memory delay (17% and 8%, respectively). The same task was performed by a human subject chronically implanted with intracortical microelectrode arrays in the fronto-parietal association cortex, which allowed us to record large-scale extracellular single-unit activity. Human behavioral and neuronal signatures were remarkably comparable to the rodent. DR trials were completed faster and more accurately than WM trials. Single neurons in the dorsolateral PFC were tuned to all investigated task variables. Importantly, the fraction of sample-selective units was again higher in the WM task than the DR task, both during sample presentation (peaking at 23% and 10%, respectively) and during the memory delay (17% and 32%, respectively). In summary, our results demonstrate that the behavioral and neuronal signatures of short-term memory differ significantly based on the current cognitive requirements and should only be cautiously generalised across tasks. Remarkably, these differences are phylogenetically conserved, underscoring the fundamental distinction of maintaining concrete behavioral choices in memory from maintaining abstract stimulus information in memory.

Disclosures: V. Hohendorf: None. **L.M. Held:** None. **R. Brini:** None. **R. Demann:** None. **A. Wagner:** None. **B. Meyer:** None. **J. Gempt:** None. **S.N. Jacob:** None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.08/Web Only

Topic: I.05. Working Memory

Title: Using cognitive event-related brain potentials to investigate spatial working memory in college students

Authors: *G. S. ROBINSON, Jr.;
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Abstract: Cognitive event-related brain potentials (ERPs) are objective non-invasive neuroelectric brain responses to specific information processing events. The purpose of this study was to investigate the utility of ERPs as objective tools to investigate spatial working memory. Eighteen college students were the participants. The average age of the participants was twenty-one. Seven scalp electrodes (Fz, Cz, Pz, C3, C4, P3, P4) were applied according to the 10-20 system of electrode placement. The tasks displayed a 3 X 3 spatial grid on a computer screen. The participants focused on the letter "X" within cells in the grid, and by pressing a button to record behavioral reaction times (RTs). Condition one consisted of 80 trials, with 20 percent of the target trials comprised of the letter "X" in the top right cell of the grid. Eighty percent of the non-targets comprised of the letter "X" in any other cells within the grid. Condition two consisted of 80 trials, 20 percent of the target trials were of the letter 'X" after the spatial sequence top right, bottom left. The non-targets were of the letter "X" in any other spatial sequence. The participants had to remember the spatial sequences to perform the task correctly. It was predicted that condition one would produce faster RTs compared to condition two, and that non-target RTs would be faster for both conditions. Furthermore, it was predicted that the ERP amplitudes will be higher for targets in both conditions. A paired-samples t-test, $t(15) = 2.40$, $p = .030$ revealed a significant difference in RTs which were faster for non-targets. Reaction times were also significantly, $t(13) = 6.89$, $p = .001$, faster for condition two targets. As for the ERP results, the central right hemisphere (scalp location C4) amplitude was significantly, $t(13) = 2.39$, $p = .03$, higher for non-targets. Additionally, higher amplitudes were revealed in the right parietal hemisphere (scalp location P4) for condition two, $t(13) = 2.87$, $p = .04$. Moreover, there was a significant higher amplitude difference for targets in the central right hemisphere (scalp location C4) for condition one, $t(12) = 3.18$, $p = .008$. The higher amplitudes to targets in both conditions revealed the right hemispheric differential processing of spatial working memory. The C4 and P4 significant results for condition two suggest the recruitment of more neuronal processes to perform a spatial working memory task. This suggests an ERP-based memory test could add more specificity to memory deficits than the traditional paper-and-pencil or computer-based memory tests. Furthermore, early objective detection of spatial working memory deficits could possibly lead to early interventions.

Disclosures: G.S. Robinson: None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.09/MM8

Topic: I.05. Working Memory

Support: NIH Grant MH132635

Title: Dopamine and serotonin concentrations in human anterior cingulate cortex scale with working memory load

Authors: *S. M. MCCLURE[1], G.-Y. BAE[2], S. R. BATTEN[4], A. COOPER[3], A. WAGLE[2], A. TORRES[2], V. JATLA[6], L. BARBOSA[5], T. M. LOHRENZ[7], R. W. BINA[1], G. BREWER[8], P. MONTAGUE[9];
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Abstract: The catecholamine neurotransmitters dopamine, serotonin, and norepinephrine have been hypothesized to gate information into working memory and support maintenance of the contents in working memory. We used machine learning-enhanced voltammetry to measure the concentration of these neurotransmitters in human anterior cingulate cortex as people performed a change detection working memory task. In the task, subjects memorized the color of one, two, or four color patches during a 1 second delay period, then reported whether a target patch changed color in a subsequent presentation. Electrochemical recordings were performed at 10 Hz, using a convolutional neural network trained on in vitro data to predict in vivo neurotransmitter concentrations. We find that dopamine and serotonin concentrations scale with working memory load during the delay period, with an opponent relationship between the two neuromodulators. These findings imply that dopamine and serotonin have complementary roles in maintaining the contents of working memory in frontal cortex.

Disclosures: S.M. McClure: None. **G. Bae:** None. **S.R. Batten:** None. **A. Cooper:** None. **A. Wagle:** None. **A. Torres:** None. **V. Jatla:** None. **L. Barbosa:** None. **T.M. Lohrenz:** None. **R.W. Bina:** None. **G. Brewer:** None. **P. Montague:** None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.10/MM9

Topic: I.05. Working Memory

Support: NCN 2022/47/B/HS6/02748

Title: Medial prefrontal cortex is involved in working memory gating: linking EEG and single-neuron activity in humans

Authors: *M. MAGNUSKI[1], A. A. KOLODZIEJ[2], K. PALUCH[1], W. M. SREDNIAWA[1], A. STANISZEWSKA[3], D. IVANOVSKI[4], R. BURNS[4], W. FORTUNA[5], K. SMARZEWSKA[5], A. BRZEZICKA[2], P. TABAKOW[5], H. BABU[4], J. KAMINSKI[1,4];
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Poland; [3]Inst. of Psychology, Polish Acad. of Sci., Warsaw, Poland; [4]SUNY Upstate Med. Univ., Syracuse, NY; [5]Wrocław Med. Univ., Wrocław, Poland

Abstract: Efficiency of the working memory (WM) system depends on flexible gating mechanisms that balance the encoding of new goal-relevant inputs with the distraction-resistant maintenance of internal representations. To investigate the neural dynamics supporting this flexibility, we examined the transition from encoding to maintenance using human EEG and intracranial recordings of single-neuron activity.
We found that uncertainty about the onset of the retention period modulates the amplitude of an EEG-derived ERP component, source-localized to medial prefrontal cortex (mPFC). Earlier—and thus more uncertain—onset of retention was also associated with a delayed increase in alpha power, a hallmark of memory maintenance. Both the ERP amplitude and alpha delay also reflected expectations shaped by memory load in previous trials. These findings suggest that unexpectedly early retention onset delays the transition to maintenance, possibly via mechanisms involving mPFC.
To validate and extend the EEG findings, we recorded single-neuron activity in epilepsy patients performing a WM task. On half of the trials, a load cue indicated the upcoming memory load, allowing participants to allocate resources effectively, leading to improved performance in the most difficult task condition. Intracranial recordings from mPFC regions identified via EEG source localization revealed complementary effects: when retention onset was unpredictable, mPFC neurons exhibited increased firing at retention onset; when a load cue was provided, mPFC activity rose in advance. Moreover, firing rates in mPFC distinguished correct from incorrect trials only after retention onset, consistent with a role in WM gate closing.
In summary, these results demonstrate that mPFC neurons facilitate the transition from encoding to maintenance in WM and that their firing patterns are mirrored in scalp EEG.

Disclosures: M. Magnuski: None. **A.A. Kolodziej:** None. **K. Paluch:** None. **W.M. Sredniawa:** None. **A. Staniszewska:** None. **D. Ivanovski:** None. **R. Burns:** None. **W. Fortuna:** None. **K. Smarzewska:** None. **A. Brzezicka:** None. **P. Tabakow:** None. **H. Babu:** None. **J. Kaminski:** None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.11/MM10

Topic: I.05. Working Memory

Support: NIH Grant R01NS129703

Title: Sensory-motor mechanisms for verbal working memory

Authors: *B. LIANG[1], A. EARLE-RICHARDSON[1], G. A. GRANT[2,3], B. FRAUSCHER[1,4,3], D. G. SOUTHWELL[4,2,5,3], G. S. HICKOK[6], G. B. COGAN[1,4,2,3,7];

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Abstract: Conversational language relies on the transient maintenance of verbal working memory (vWM), yet the precise neural mechanisms supporting vWM remain elusive. To address this gap, in this study, we recorded and analyzed neural signals from patients implanted with stereoelectroencephalography (sEEG) electrodes performing a lexical repetition task with a vWM delay. 37 patients (mean age = 31, 21 female) with electrodes implanted as part of their surgical epilepsy evaluation were recruited in this study. In each trial, patients heard a word or nonword (e.g., "bacon", "valuk"), and repeated it aloud after a visual Go cue following a randomized delay. As a control analysis, 18 of the recruited patients completed another lexical repetition task with the same stimuli but without delay. Local neural engagement was identified as significant increases in high-gamma power (HG, 70-150 Hz) relative to a pre-trial baseline (permutation t-test, $p < .05$; time cluster-based correction, $p < .05$). We found Delay electrodes (N = 1009) with significant HG responses during vWM delay. Within the Delay electrodes, 33.11% (N = 402) also had auditory responses (Auditory delay electrodes), 20.51% (N = 249) also had motor responses (Motor delay electrodes), and 38.06% (N = 462) also had both responses (Sensory-motor delay electrodes). We also found electrodes with only delay activity (Delay-only electrodes, 7.33%, N = 89). Moreover, we analyzed the subset of patients with the control task and found that Delay electrodes with auditory and motor responses remained active even without delay (auditory: 79.22%; motor: 67.67%), but the Delay-only electrodes mostly became silent (78.57%). These results indicate that vWM activity largely stems from general sensory-motor speech processing, even without an explicit vWM task, but vWM-specific activity also exists. Motor and Auditory delay electrodes were distributed in the dorsal (frontoparietal) and ventral (temporal) streams of speech processing, respectively, while the Sensory-motor delay electrodes were spatially adjacent to both electrode types, spanning both processing streams. Delay-only electrodes were distributed in the frontoparietal network subserving executive functions. These results suggest that the general sensory-motor neural activity supports vWM maintenance by linking receptive and generative speech processing, along with a vWM-specific executive module. Taken together, we found that vWM is supported by general sensory-motor speech processing neural mechanisms with potential vWM-specific control processes.

Disclosures: B. Liang: None. **A. Earle-Richardson:** None. **G.A. Grant:** None. **B. Frauscher:** None. **D.G. Southwell:** None. **G.S. Hickok:** None. **G.B. Cogan:** None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.12/MM11

Topic: I.05. Working Memory

Title: Semi-orthogonal operand sub-spaces in the hippocampus, anterior cingulate cortex underpin flexible sequential arithmetic

Authors: *S. HAM[1], H. SOHN[2];
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Abstract: For arithmetic operations, neural circuits must preserve each operand's identity until the pending computation is executed. Here we ask how the human cortex accomplishes such separation. Building on recent neuroeconomics findings showing that semi-orthogonal subspaces bind offer values to choice actions, we hypothesized that analogous population geometry supports binding two sequential numeric operands. Here we ask whether the human cortex implements this geometry. To probe how operator timing shapes operand storage, we contrasted prefix trials (e.g., "+ 3 5"), in which the rule is known before the numbers, with postfix trials (e.g., "3 5 +"), in which the rule appears afterward—testing conditions where operands can be preassigned versus held neutrally until the operator emerges.During task performance, we recorded single-neuron activity from multiple cortical areas—including the hippocampus, entorhinal cortex, anterior cingulate cortex, and orbitofrontal cortex—in neurosurgical patients (N=6) as they solved one hundred trials of prefix/postfix arithmetic problems. At the single-cell level, a significant proportion of neurons in ACC (10.5%) and HPC (10.8%) exhibited number-selective tuning. In both regions, preferred numerosities often shifted or vanished across epochs, demonstrating context-sensitive coding. This dynamic tuning indicates that individual neurons concurrently encode operand identity and temporal context, enabling the population to maintain segregation of successive operands over time.We further examined the properties of the population through neural subspace analysis. Specifically, we hypothesized that the subspace would be orthogonalized to prevent interference between the representations of successive operands. Indeed, we found that each operand occupied a distinct, semi-orthogonal subspace, with clear divergence according to operand identity. This geometric arrangement prevented overlap of operand representations and ensured that each operand remained in its subspace, whether the operator was known in advance (prefix) or only afterward (postfix). Together, these findings offer insights into the neural mechanisms of arithmetic operation and highlight how partially orthogonal population codes support dynamic operand processing in the human cortex.

Disclosures: S. Ham: None. **H. Sohn:** None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.13/MM12

Topic: I.05. Working Memory

Support: DFG JA 1999/6-1

Title: Flexible representation of symbolic and non-symbolic numerical information in working memory by human prefrontal neurons

Authors: *H. CHEN[1,2,3], X. SHAN[1,2], L. SCHIFFL[1,2], G. ALKAN[1,2], A. WAGNER[2], B. MEYER[2], J. GEMPT[4], S. N. JACOB[1,2];
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Abstract: Working memory is crucial for cognitive flexibility in the prefrontal cortex (PFC), enabling context-dependent decision-making. Numerical processing has been shown to strongly drive the PFC. However, whereas non-symbolic representations are shared across species, processing symbolic numbers is uniquely human, indicating advanced reasoning. To explore the neuronal mechanisms underlying flexible representation and retrieval of symbolic and non-symbolic content in working memory, we recorded large-scale spiking activity (n = 2488 well-isolated single-neurons) from microelectrode arrays chronically implanted in the right middle frontal gyrus (MFG) and inferior frontal gyrus (IFG) of a human subject performing number comparison tasks with retro-cueing. The subject was required to memorize two sample stimuli, presented either sequentially (temporal context) or simultaneously (spatial context), and retrieve one of the samples indicated by a retro-cue for comparison with a test stimulus. Numerical quantities served as stimuli and were presented in either non-symbolic notation (sets of dots, numerosities) or symbolic notation (digits, Arabic numerals). In the first experiment, the subject judged whether the cued number matched the test number. A substantial proportion of neurons in both the MFG (~30%) and IFG (~38%) encoded numerical information across maintenance and retrieval epochs. Surprisingly, despite high behavioral accuracy for both notations, significantly fewer neurons were tuned to symbolic than to non-symbolic numbers across all epochs and tasks. We hypothesized that Arabic numerals might have been processed primarily as visual shapes without accessing magnitudes, as the task only required a match/non-match comparison. To test this hypothesis, we conducted a second experiment in which the subject judged whether the cued number was larger than the test number, enforcing the comparison of magnitudes in both notations. Again, we observed robust tuning to non-symbolic numbers and minimal tuning to symbolic numbers. These consistent findings suggest that processing symbolic information in the human association cortex might involve alternative coding strategies, such as sparse and resource-efficient coding. Overall, our results show that the notation of memoranda, i.e., the level of symbolic reference, significantly influences neuronal representations for working memory maintenance and flexible retrieval.

Disclosures: H. Chen: None. **X. Shan:** None. **L. Schiffl:** None. **G. Alkan:** None. **A. Wagner:** None. **B. Meyer:** None. **J. Gempt:** None. **S.N. Jacob:** None.

Poster

PSTR411: Central and Prefrontal Mechanisms of Working Memory in Humans

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR411.14/MM13

Topic: I.05. Working Memory

Support: ONE Munich Strategy Forum

Title: Single-neuron encoding of symbolic and non-symbolic working memory content in the human frontoparietal cortex

Authors: *P. FAVERO[1,2], L. SCHIFFL[1,2], B. TASCI[1,2], G. ALKAN[1,2], L. M. HELD[1,2], H. CHEN[1,2], A. WAGNER[2], B. MEYER[2], J. GEMPT[3], S. N. JACOB[1,2];
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Abstract: The use of symbols to convey meaning is a hallmark of human cognition. It is not known whether symbolic and non-symbolic information is processed via shared neuronal mechanisms. We present a unique case study of large-scale extracellular microelectrode recordings with single-neuron resolution from the frontoparietal association cortex of an individual with stroke-induced language production impairments (non-fluent aphasia) and largely preserved language comprehension and non-linguistic cognitive functions. We administered two delayed match-to-sample tasks using non-symbolic stimuli (icons) and symbolic stimuli (written/spoken words) drawn from eight semantic categories. In the perceptual matching task (PM), the participant judged whether test and sample stimuli matched based on sensory features; in the semantic matching task (SM), they determined whether the stimuli belonged to the same semantic category, requiring access to meaning. Behavioral performance was near ceiling in PM while it varied across stimulus reference levels in SM: icons (93% one-sample, 85% two-sample), written words (88%, 76%), and spoken words (81%, 66%). A significant interaction between task and reference level was found ($p < 1e-13$), suggesting that SM accuracy differences reflect higher demands for symbolic processing rather than working memory load. Across eight sessions (one-sample), 1346 single and 479 multi-units were recorded from the right-hemispheric middle frontal gyrus (MFG), inferior frontal gyrus (IFG), supramarginal gyrus (SMG) and angular gyrus (ANG), areas linked to semantic processing and working memory. Of these, 1290 units were task-modulated, with 56% exhibiting mixed-selectivity to the task factors, and the rest being temporally modulated but unselective. A subset of mixed-selective neurons showed increased spiking for specific stimulus properties, either sensory modality (5%) or reference level (2%). Using cross-temporal linear discriminant analysis classification on trial-wise spike rates, we found that task identity was most accurately decoded from MFG and IFG activity after test stimulus onset, as the task rule was applied. Decoding analyses also revealed sustained neuronal representations of both stimulus modality and reference level during stimulus presentation and throughout the delays. Together, our findings suggest that symbolic and non-symbolic content can be encoded by frontoparietal working memory circuits. Further research is needed to clarify the extent to which semantic representations are shared across symbolic and non-symbolic notations.

Disclosures: P. Favero: None. **L. Schiffl:** None. **B. Tasci:** None. **G. Alkan:** None. **L.M. Held:** None. **H. Chen:** None. **A. Wagner:** None. **B. Meyer:** None. **J. Gempt:** None. **S.N. Jacob:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.01/MM14

Topic: I.07. Long-Term Memory

Support: Internal Department Funding

Title: Noise correlations in human hippocampal neurons contain information relevant for episodic memory

Authors: *M. SIVAKUMAR, B. C. LEGA;
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Abstract: Neuronal trial to trial co-firing variability for a given stimulus, or noise correlations, may influence information encoding and memory behavior. We tested if their geometry differs between correct/incorrect human episodic memory. We analyzed single-unit activity from drug-resistant epilepsy patients performing associative recall (AR, word pair classification: intact, rearranged, novel) and serial recall (SR, 10-word list order recall). We analyzed SR encoding in 10 subjects (3 with early [1-4] vs. late [7-10] word positions) and AR encoding/retrieval and Correct vs. Novel in 4 subjects. For each neuron pair, trial-to- trial firing rates were plotted in 2D. We extracted two metrics: signal-noise angle (angle between signal axis, which connects mean activity between correct and incorrect/novel, and the noise axis, the first eigenvector of covariance matrix for correct and incorrect/novel trials); and major axis length (co-firing spread along noise axis for the correct and incorrect/novel trials). We hypothesized correct trials would show larger angles, as greater signal-noise orthogonality aids effective encoding. Signed-rank and Rank-sum tests (all $p<0.001$) revealed significant differences across conditions. For signal-noise angles (values are mean ± SD), AR encoding showed Correct 37.6° ± 2.9° vs. Incorrect 41.8° ± 3.1°, AR retrieval showed Correct 37.3° ± 3.2° vs. Incorrect 33.9° ± 3.2°, Correct vs. Novel (AR) was 39.3° ± 2.8° vs. 36.1° ± 2.5°, and SR late encoding was Correct 31.9° ± 2.7° vs. Incorrect 27.3° ± 2.4°. Signal-noise angle differences were also significant between AR and SR encoding for correct (SR Correct: 32.3° ± 3.0°) and incorrect (SR Incorrect: 34.5° ± 2.9°) conditions, and angle differences were further confirmed between SR early (26.8° ± 4.2° Correct, 28.4° ± 4.1° Incorrect) vs. SR late conditions, as well as AR encoding vs. retrieval for incorrect. Major axis length also significantly differed ($p<0.001$), with greater variability in SR late showing Incorrect 9.55 ± 1.0 vs. Correct 8.55 ± 0.6, and SR early showing Correct 12.4 ± 1.5 vs. Incorrect 9.7 ± 0.7. Greater signal-noise separation generally correlates with successful memory particularly in AR retrieval and SR late encoding. The inverse pattern in AR encoding implies task-specific neural dynamics. Correct AR retrieval demonstrated enhanced discriminability

from novel items. Higher angles in late SR correct trials suggest evolving neural representations over time. Additionally, co-firing variability may aid early encoding but disrupts late encoding. These findings indicate that hippocampal noise correlations encode information relevant to episodic memory in humans.

Disclosures: M. Sivakumar: None. **B.C. Lega:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.02/MM15

Topic: I.07. Long-Term Memory

Support: Natural Sciences and Engineering Research Council of Canada Discovery Grant RGPIN-2023-04231
Canada Foundation for Innovation JELF and Ontario Research Fund 34479
Canada Research Chair CRC-2019-00107
New Frontiers Research Fund NFRFE-2019-508778

Title: Hippocampal theta phase drives rhythmic memory formation

Authors: *T. M. BIBA[1], Q. CHU[2], A. CORNEJO MARIN[3], I. SKELIN[3], T. A. VALIANTE[3], K. D. DUNCAN[1];
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Abstract: Episodic memory formation is hypothesized to occur at a specific phase of hippocampal theta oscillations (3-10 Hz), when synaptic plasticity, neural inhibition, and perceptual drive dominates (Hasselmo et al., 2002). Our recent work adapted a dense sampling approach, demonstrating the probability of human memory formation indeed fluctuates at theta (Biba et al., 2024). However, it remains to be determined if rhythmic boosts in memory occur at a particular hippocampal theta phase. Here, we collected intracranial (iEEG) recordings from individuals with medically refractory epilepsy (n=15) who performed the mnemonic dense sampling task. Specifically, on each trial, we presented an attention-grabbing cue designed to reset neural oscillations to a consistent phase across trials. We then varied the stimulus onset asynchrony (SOA) between the cue and a trial-unique object image (300-1200ms) during encoding, to construct a time-course of subsequent associative memory as a function of encoding SOA. Critically, if hippocampal theta phase is consistent across trials, the timing of object presentation at each SOA will correspond to a specific neural phase. First, we verified the cue re-organized hippocampal theta phase, with inter-trial clustering (ITC) analysis, for trials with SOA's greater than 900ms (3-10 Hz, p = 0, 0-900ms post cue). Next, behavioral spectral analysis confirmed that the probability of iEEG participants' memory formation oscillated at theta

(7.23±3.01 Hz, $p < 0.05$) as a function of the cue-image encoding SOA, at a frequency consistent with our prior large cohort (7 Hz, n=125; Biba et al., 2024). To link these behavioral memory rhythms to hippocampal theta phase, we adapted an established approach (Feibelkorn et al., 2018) used to study attention rhythms, to hippocampal memory. First, we averaged subsequent associative memory success based on pre-image hippocampal theta phase during encoding, and quantified phase-behavior amplitude using spectral analysis. Permutation testing in all hippocampal electrodes (n=82) yielded significant phase-behavior relationships at the group level, with an average frequency that closely matched behavioral memory rhythms in iEEG participants (7.8±3.2 Hz, $p<0.05$, Amplitude=12.5%). Our results suggest that hippocampal theta phase at encoding influences the fate of subsequent associative memory, rhythmically sampling information into memory seven times per second.

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Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.03/MM16

Topic: I.07. Long-Term Memory

Support: Seale Innovation CY25 - 452136

Title: Encoding and Retrieval Mnemonic Discrimination in the Medial Temporal Lobe - Implications for Ultrasound-Targeted Memory Neuromodulation

Authors: *Y. NI[1,2,4], M. NIMKAR[6], W. LEGON[4,3,7,5];
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Abstract: The medial temporal lobe (MTL) is crucial for episodic memory, yet its specific contributions to mnemonic discrimination—the ability to differentiate previously encoded items from similar lures—across memory encoding and retrieval phases are not well understood. While previous research has linked MTL engagement to either encoding or retrieval, few studies have directly compared how distinct MTL subregions process mnemonic content within individuals across these phases. Understanding this is vital for guiding precise memory-enhancing interventions, such as noninvasive low-intensity focused ultrasound (LIFU). In this study, we analyzed functional magnetic resonance imaging (fMRI) data from seven human participants (5 male; mean age = 26.0 ± 6.1 years) who performed a face-name associative memory task with both encoding and recognition phases, including confidence judgments. Event-related general linear models were used to capture trial-wise memory activity. To investigate the interaction

between memory phase and accuracy, we used a second-level group contrast that assessed the difference in activity between retrieval hits and lures, compared to the difference between encoding hits and lures. Region-of-interest analyses revealed that the left posterior hippocampus and bilateral parahippocampal cortex, showed significantly greater mnemonic discrimination-related activity during successfully retrieval compared to encoding ($p<0.05$). These findings suggest that memory-relevant computations like pattern completion and contextual reinstatement are selectively amplified in posterior MTL subregions during retrieval. We demonstrate that the modulation of mnemonic specificity is specific to particular subregions of the medial temporal lobe and to the retrieval process. This offers a nuanced understanding of episodic memory's functional organization. Importantly, these findings suggest that the distinct activation patterns of these subregions could serve as a more effective functional biomarker for targeted neuromodulation strategies—such as applying LIFU to the posterior hippocampus during retrieval—to enhance memory discrimination.

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Poster

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Program #/Poster #: PSTR412.04/MM17

Topic: I.07. Long-Term Memory

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Title: Precision and binding within hippocampal subfields as determined using high-resolution hippocampal fMRI

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Abstract: Substantial evidence supports a critical role for the hippocampus in episodic memory, our ability to recall events and their spatiotemporal contexts. However, emerging evidence suggests that this role might extend outside of the classic domain of memory and include aspects of perception and short-term memory. As such, the hippocampus may serve a broad role in supporting representations that are fine-grained and multidimensional - including but not restricted to episodic memory. To test this, We recruited 23 young healthy adults to participate in a source-memory task while placed in a 3T MRI scanner, in which words were presented on-screen sequentially and in varying colors and spatial locations. Participants were instructed to memorize words as well as their corresponding spatial locations and color attributes. Participants were later tested on their memory of words (old/new recognition) and their fine-grained recall of space/color source-memory using a continuous response-dial. The experiment featured 3 blocks

that tested for either spatial position and or color in isolation (unidimensional) and 3 blocks that tested for both (multidimensional). If the hippocampus and connected regions are involved in both precision and binding, then contrasts relevant to precision (low vs. high degree error) and binding (uni- vs. multidimensional) should reveal voxels centered in the hippocampus. We performed high-resolution hippocampal imaging, including a multiband EPI sequence designed to capture task-relevant BOLD in the hippocampus. Participants performed well above chance at item recognition task (85% Hit+CR rate) and also performed above chance at the source-memory tasks (average 16 deg. offset for recognition hits). There was no statistical difference in degree offset for color vs. space ($t(22)=-1.19, p=.24$) or in uni- vs. multidimensional blocks ($t(22)=.71, p=.48$). A whole-brain contrast at the first level (Recognition-Hit - Simple-Motor) revealed significant ($p<.001$) clusters of 10+ voxels in ant. temporal lobe and fusiform gyrus. A pilot contrast modelling degree-offset using a parametric modulator revealed a cluster of voxels in the retrosplenial cortex ($p<.001$), a region that may support reinstantiation of complex stimuli in conjunction with the hippocampus. We expect that ongoing analysis at the second level will reveal functionally distinct sites within the hippocampus and surrounding medial temporal lobe that are important for item-binding and precision. These data will advance our understanding of how functionally distinct subregions within human temporal support rich multidimensional representations.

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Title: Concept cells in the temporal pole: a neuronal code for semantic knowledge

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Abstract: Single-neuron recordings in humans provide a unique opportunity to evaluate cognitive models at the neuronal level [1,2]. Intracerebral recordings have identified neurons

selective to one particular concept, termed *concept cells* [3], in the hippocampus and adjacent medial temporal lobe (MTL) structures as the building blocks of declarative memory [4]. However, these concept cells demonstrate strong semantic properties, such as multimodal responses [5] and coding for broad semantic category [6], rather than episodic properties [7]. Finding such selective neurons within the hippocampus and MTL structures presents a paradox given that it is the *temporal pole* (TP) that is widely regarded as the primary hub for semantic memory [8]. This raises critical questions regarding the type of neuronal code underlying semantic knowledge in the TP and its functional relationship with the neuronal code observed in the hippocampus. To investigate this issue, we recorded the activity from 539 single units across 16 patients implanted with tetrodes [9] from the TP (n=129), MTL (n=261), and other brain regions (n=149), while they watched series of pictures (see [3,10] for methodological details). Among these, 80 single units in the temporal pole of five different patients exhibited increased activity to at least one concept. At the single-unit level we observed highly selective cells. These units displayed several defining characteristics: (1) invariant responses to different exemplars of the same concept, (2) response latencies shorter than those observed in the MTL (approximately 250 ms post-stimulus onset), (3) multimodal encoding of conceptual information, (4) explicit representation of concepts, (5) a less sparse code than in the hippocampus and (6) absence of responses to non-semantic stimuli. Preliminary neural manifold analyses [11]revealed a neuronal code for broad semantic category with different time trajectories between semantic categories, revealing a dual-level code: at the single neuron level for specific concepts and at the population level for broad semantic category. These findings provide novel insights into the neuronal coding of semantic concepts. Specifically, they suggest that semantic concepts are represented by highly specialized neurons within the TP, akin to the coding mechanisms observed in the hippocampus and MTL. A key question emerging from these results is the functional significance of such a distributed semantic coding scheme across multiple, anatomically distinct brain regions. Further investigation is required to elucidate the mechanisms underlying this distributed representation and its implications for the organization of semantic memory.

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Poster

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Title: Auditory and multisensory representations in the human hippocampus

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Abstract: The human hippocampus is traditionally considered a brain region dedicated to long-term memory. An emerging literature suggests that it may also contribute to several aspects of visual perception, including eye movements, mental imagery, scene discrimination, and visual search. However, the hippocampus and surrounding medial temporal lobe (MTL) cortex receive anatomical inputs from virtually all sensory systems, raising the question of how the hippocampus might contribute to perception of sensory modalities beyond vision and to the multisensory combination of visual and other modalities. We conducted a high-resolution fMRI study (N = 30) to understand the distribution of unisensory (visual and auditory) and multisensory representations in automatically segmented hippocampal regions of interest. Participants were exposed to multiple repetitions of 10 dynamic movie scenes (6s in duration) presented in distinct formats: purely auditory, purely visual, congruent audiovisual, and incongruent audiovisual (audio and video from different scenes). We applied multivariate pattern classification and representational similarity analyses to decode the identity of movies within hippocampal subfields and MTL cortical subregions. Unisensory visual and auditory movies were represented reliably in the CA1 subfield, as well as in the subiculum, perirhinal cortex, and parahippocampal cortex. In contrast, CA2/3 and entorhinal cortex represented the audiovisual movies, but only when congruent. These findings provide the initial evidence for auditory and multisensory coding in the human hippocampus during perception, in the absence of explicit memory demands, and suggest a dissociation in the role of different hippocampal subfields. Future work will link these perceptual representations to the formation of multisensory memories.

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Poster

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Title: Relational memory encoding in the human infant hippocampus

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Abstract: Episodic memories are fundamentally relational, with people and objects bound to moments in time and locations in space. It is unclear how and when the ability to encode such relations develops in the human brain. Using awake infant fMRI, we recently discovered that the hippocampus, especially the posterior portion, can encode individual items after a single exposure by 12 months of age. Here, we test whether this early hippocampal function extends to memory for relations between items. We have collected a partial sample of fMRI data from 18 awake and behaving infants (4-23 months of age) in a classic face/scene relational binding task. Infants viewed a series of encoding trials that began with a dynamic video of a landscape scene on which a dynamic video of a face was superimposed. We interleaved test trials among these encoding trials, with the test trial for a particular face-scene pair appearing approximately 60 s on average after its encoding. During each test trial, the infants were cued with a familiar scene after which two equally familiar faces appeared side-by-side, creating a visual paired comparison between a matching face that had been paired with the scene during encoding and a non-matching face that had been paired with a different scene. Preferential looking to the matching face, measured with an MRI-safe camera, indicated that the infant successfully bound the face-scene relation during encoding. We hypothesized that activity in the hippocampus would be greater during encoding of these subsequently remembered relations, relative to relations that were not remembered, especially in older infants and in the posterior hippocampus. To test these hypotheses, we examined BOLD activity in a bilateral hippocampal region of interest defined using infant-trained automated hippocampal segmentation, which was then manually divided into anterior (head) and posterior (body and tail) portions. Preliminary results support these hypotheses, suggesting that the hippocampus begins to support even sophisticated episodic memory abilities during infancy. These findings are consistent with the theory that infantile amnesia, our lack of memories from infancy later in life, arises from a failure of retrieval rather than a failure of encoding.

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Topic: I.07. Long-Term Memory

Support: NIH R01 AG082680

Title: Associations between age, memory performance and hippocampal and medial temporal sub-regional volumes

Authors: *A. S. ANANDAN[1], M. J. RICCO[2], M. A. DE CHASTELAINE[4], A. KIDWAI[3], M. D. RUGG[2];
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Abstract: Previous studies have reported mixed findings when examining associations between hippocampal (or hippocampal subfield) volume and memory performance in young adults. Here, we employed Automated Segmentation of Hippocampal Subfields (ASHS) to examine associations between memory performance and the volume of hippocampal subfields and surrounding medial temporal lobe (MTL) neocortical regions in a sample of young adults. Thirty-five cognitively healthy participants aged 18-27 years (mean: 21.86; 18 female) were administered a comprehensive battery of neuropsychological tests that included the California Verbal Learning Test (CVLT). In a separate MRI session, T1-weighted and high-resolution T2-weighted MRI scans of the brain were collected as part of a functional imaging study that required participants to perform an experimental test of source memory. Structural images were processed via the ASHS analysis pipeline to obtain volumetric data for hippocampal subfields (left and right CA1, CA2/3, dentate gyrus (DG) and subiculum) and surrounding MTL regions (left and right entorhinal, perirhinal and parahippocampal cortex; ERC, PRC, PHC, respectively), as defined by the Princeton Atlas. All volumes were adjusted for intra-cranial volume (ICV). Strikingly, the left and right PHC volume were the only regions to show a strong negative correlation with chronological age ($r > -.400$). After controlling for age, significant positive correlations were identified between right CA1 and right DG volume and source memory performance on the in-scanner retrieval task ($rs > .340$). Conversely, reliable negative correlations were identified between both short and long delay CVLT free recall and the volume of the left ERC ($r = -.409$ for recall collapsed across delay). Additionally, left ERC volume correlated positively with CVLT recognition false alarms ($r = .355$). The findings for the PHC suggest that this region is especially sensitive to the cross-sectional effects of age in young adulthood, even within a restricted age range as was the case here. The negative associations between performance on the CVLT and left ERC volume are reminiscent of prior findings reported in young adults of associations between different metrics of cognitive performance and both whole brain and hippocampal structural measures.

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Title: Representation of visual sequences in the tuning and topology of neuronal activity in the human hippocampus

Authors: *T. JOHN[1], Y. ZHOU[2], A. ALJISHI[3], B. RIECK[4], N. B. TURK-BROWNE[5], E. C. DAMISAH[6];
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Abstract: The hippocampus plays a critical role in the formation of memories and in the representation of time, yet the coding principles that connect these functions are poorly understood. Prior work in primates showed that repeated training on visual sequences alters the tuning of inferior temporal neurons based on temporal distance from a preferred stimulus, while recent fMRI and intracranial studies in humans show increased pattern similarity in hippocampus for statistically learned temporal pairs. These studies suggest a general coding principle in which human hippocampal neurons adjust their tuning to visual stimuli in a distance-dependent manner to represent sequence structure. To test this principle, we investigated how temporal regularities influence the tuning and population activity of neurons recorded with microwires in 17 subjects with epilepsy undergoing intracranial study. Participants watched an initially arbitrary sequence of photographs of natural scenes in blocks where the order of the scenes was fixed across sequence repetitions (structured condition) or scrambled on each repetition (random condition). These conditions were otherwise identical in the type, number, and frequency of individual stimuli. Hippocampal neurons showed increased firing to stimuli near to their preferred stimulus in the structured sequence and decreased firing to more distal stimuli in that sequence. This modulation of tuning by temporal distance was absent for the random sequence and in control brain regions. Topological data analysis revealed that these neuronal changes correspond to a population code for the global geometry of the learned sequence, with hippocampal activity embedding the (looping) structured sequence as a high-dimensional ring in its representational space. These findings contribute to a more comprehensive understanding of hippocampal function in the integration of objects, time, and space, demonstrating that learning of temporal regularities can modify the tuning of hippocampal neurons to arbitrary visual features. This coding for sequence structure enables efficient prediction and behavior and could serve as a shared foundation for statistical learning and episodic memory.

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Poster

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SPP 2411 (PN 520287829), MO 930/4-2

Title: Decoding movie content from neuronal population activity in the human medial temporal lobe

Authors: *A. DARCHER[1], F. GERKEN[2], P. J. GONCALVES[3], R. RAPP[4], I. ELEZI[2], J. NIEDIEK[5], M. S. KEHL[6], T. P. REBER[7], S. LIEBE[8], J. H. MACKE[4], F. MORMANN[1], L. LEAL-TAIXE[2];
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Abstract: The human medial temporal lobe (MTL), a region implicated in memory and high-level cognition, contains neurons that respond selectively to stimuli belonging to specific categories, such as individual people, landmarks, or objects. However, these neurons have been largely studied via static, isolated presentations of stimuli. It is therefore unclear how neurons in the MTL respond to rich stimuli such as movies, and which dynamical stimulus features can be retrieved from neuronal population spiking activity. We studied single-unit responses from 2286 neurons recorded from the amygdala, hippocampus, entorhinal cortex, and parahippocampal cortex of 29 intracranially implanted patients (17 female; ages 22-63) during the presentation of an 83-minute movie. We found only a few individual neurons that exhibited a classic selective response to semantic features, such as the main character of the movie (78 units; $p=0.033$, cluster permutation test) and the general on-screen appearance of persons (4, $p<0.001$). Most individually selective neurons responded to the occurrence of visual transitions such as camera cuts (746, $p<0.01$) and scene cuts (81, $p<0.05$). Despite the absence of stimulus-selective responses for most features on the single-unit level, we successfully decoded the presence of main characters, setting, and the occurrence of visual transitions from the activity of the neuronal pseudo-population aggregated across subjects. We compared decoding performance between recurrent and linear models and found that visual transitions rely on the temporal information in neural sequences while character-related information does not. We then examined which neuronal subsets contributed most to the decoding performance and found that the relevant information varies across regions depending on the feature category. Visual transitions largely depended on the activity of individually responsive neurons in the parahippocampal cortex, whereas character and location features relied on distributed representations not apparent in the spiking of discrete units. Our results demonstrate an approach for reliably decoding movie features in the human MTL and suggest that the brain uses a population code when representing character and location features, but a sparser code when representing visual transitions.

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Poster

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Support: SFB 1089

Title: Visually selective neurons in the human medial temporal lobe are modulated by attention

Authors: *I. VIETEN[1], J. FABER[2], V. BORGER[3], R. SURGES[1], F. MORMANN[1];
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Abstract: Structures of the medial temporal lobe (MTL), most notably the hippocampus along with its input and output regions, play a key role in the formation of long-term memory. Attention is a key mechanism through which perceptual input is tagged for long-term memory consolidation. The human MTL hosts neurons that selectively and invariantly respond to the semantic content of presented stimuli. These neurons are called concept cells, and they have been shown to constitute the semantic building blocks of human episodic memory. An open question is how the hippocampal system uses attention to select which stimulus responses to reinforce and sustain. We here recorded single-neuron activity from different regions of the MTL in epilepsy patients undergoing invasive seizure monitoring. We targeted concept cells by presenting their preferred stimuli embedded in a rapid series of 8 different images. The patient's task was to count the occurrence of a particular image in each experimental run, thus guiding their attention to each of the presented stimuli in turn. We recorded visually selective cells in the parahippocampal cortex (PHC, n = 58), the hippocampus (n = 23), and the amygdala (n = 43). When comparing concept cell responses to preferred stimuli presented as target vs. distractor, we found a significant increase in median response magnitude in the PHC and an increase in response duration in the PHC and the amygdala. Interestingly, the number of trials during which the preferred stimulus elicited no response at all was around 50% across all regions of the MTL. This percentage, however, was modulated by attention. Both in the hippocampus and in the PHC, a significant number of units showed a greater proportion of silent trials in the distractor than in the target condition. In the amygdala, 6 neurons showed a greater proportion of silent trials for target vs. distractor presentations, while 7 neurons showed the opposite effect. However, the percentage of silent trials was not the relevant parameter underlying the attentional modulation of median response magnitude. When only considering active trials, an increase in median response magnitude was found not only in the PHC, but also in the hippocampus. Our results suggest that attentional mechanisms affect PHC and hippocampal units similarly, broadly

increasing their sensitivity to preferred stimuli. Amygdala units are more diversely modulated, possibly owing to their localization in different subnuclei, which we cannot spatially resolve.

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Topic: I.07. Long-Term Memory

Support: CSC scholarship

Title: Fear conditioning and extinction modulates single unit activity in the human medial temporal lobe and prefrontal cortex

Authors: *Y. QIN[1], V. BORGER[2], R. SURGES[1], D. R. BACH[3], F. MORMANN[1];
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Abstract: Many animal studies indicate that fear acquisition and extinction mainly rely on the amygdala and prefrontal cortex, specifically a small number of sparsely distributed neurons modulate both processes by changing their firing rates (FR). Despite many human neuroimaging studies, due to low spatial resolution, the single-unit correlates of fear conditioning and extinction in humans have remained poorly understood.
Here, we recorded single-unit activity from human subjects implanted with depth electrodes for chronic seizure monitoring in the medial temporal lobe (MTL) and prefrontal cortex (up to now 992 units from 6 patients). One of two presented stimuli (images) was paired with aversive unconditioned stimulus (electrical shock/noise).
By comparing the FR during 3 experiment phases (habituation, conditioning and extinction) at the single-unit level, we identified 4.5% neurons which changed FR after conditioning. The frontal cortex showed the highest fraction (8%), followed by MTL subregions (amygdala, hippocampus, parahippocampal and entorhinal cortex, all ~ 4%), results from other areas did not exceed chance level. These neurons were further split into 2 subgroups, ON and OFF, each corresponding to significantly higher or lower FR during conditioning compared to habituation phase. We observed more OFF than ON cells in the amygdala, entorhinal and frontal cortex. Similarly we found 2.3% of neurons which changed their FR after extinction compared to after conditioning. Here, only the fraction of hippocampal neurons (2.5%) exceeded chance level, with no significant difference between ON and OFF cells.
Together, we provided the first description of single-neuron correlates of fear learning and extinction in the human brain and showed a distinct involvement of the MTL and prefrontal cortex.

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Title: Compositional representation of letter strings in human MTL neuron firing

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Abstract: The medial temporal lobe (MTL) is critical for human memory, yet it is unclear how an abstract symbol is encoded. We recorded single neuron activity from the MTL of 9 epilepsy patients implanted with depth electrodes while they performed a working memory task where letter strings of set size 4, 6, or 8 were presented. Letters were selected from 15 consonants and shown during the encoding stage (duration 2 s), followed by the maintenance stage (3 s) and visual presentation of a probe letter, which initiated the retrieval stage. Across participants, we recorded 1200 well-isolated neurons. To uncover low-dimensional structure, we applied singular value decomposition (SVD) to neural firing and aligned components across subjects and sessions. We retained 19 principal components (74% explained variance) with correlated population dynamics across individuals during task performance. Time decoding in the component space revealed stage-specific dynamics: encoding and retrieval showed rapidly evolving activity, while maintenance showed more persistent patterns, consistent with stable internal representations. We modeled residual dynamics, defined as trial-by-trial fluctuations around mean trajectories, using linear models. Eigenvalue distributions indicated more persistent dynamics during maintenance than during encoding or retrieval. To assess compositional coding, we trained linear decoders to identify letter identity in the 19-dimensional neural subspace. The set of decoders spanned a 15-dimensional subspace, corresponding to the number of 15 letters. The set of decoders remained stable between the encoding stage and the maintenance stage. During the encoding, maintenance and retrieval stages, the single-trial activity was preferentially projected onto the axes of presented letters (>52% vs. 50% chance level, p-value < .005). Decoders trained on set sizes 4 and 6 generalized to set size 8, indicating representational stability across memory load. After presentation of the probe letter during retrieval, projections identified the probe letter ($p = .018$). We thus mapped MTL neuron firing onto a low-dimensional space that allows the representation of abstract symbolic content.

Disclosures: F. Costa: None. **D. Ledergerber:** None. **J. Sarnthein:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.14/NN9

Topic: I.07. Long-Term Memory

Support: SFB 1089

Title: Single-neuron correlates of conscious perception during peripheral vision in the human medial temporal lobe

Authors: *N. KRENN[1], V. BORGER[2], R. SURGES[1], F. MORMANN[1];
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Abstract: The more peripheral in the visual field a stimulus is presented, the harder it becomes to detect it and to consciously perceive a displayed item. It is unclear whether human concept neurons respond to different levels of detectability in a graded or an all-or-nothing manner. Up to now, we have recorded from 3827 units in the medial temporal lobe (including amygdala, hippocampus, entorhinal cortex and parahippocampal cortex) across 41 experimental sessions from 9 neurosurgical patients implanted with hybrid Behnke-Fried depth electrodes. In each session we displayed stimuli at eccentricities of 0, 5, 10, and 15 degrees of visual angle, and performed simultaneous eye-tracking. Subjects fixated centrally and reported the concept they perceived across the presented eccentricities. The analysis of our preliminary dataset reveals that the firing rates of concept cells are not only higher for detected versus undetected concepts, but that the firing rates in response to detected concepts also decrease with increasing eccentricity. In addition, the response latencies of these neurons show an increase with increasing eccentricity for detected concepts. Furthermore, the concept cells displayed higher firing rates in response to undetected preferred versus undetected non-preferred stimuli. These findings suggest that both firing rates and neuronal response latencies exhibit a graded relationship with the detectability of preferred concepts in the peripheral field of view, providing insight into how conscious perception emerges in the brain during peripheral vision.

Disclosures: N. Krenn: None. **V. Borger:** None. **R. Surges:** None. **F. Mormann:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.15/NN10

Topic: I.07. Long-Term Memory

Support: SFB 1089

Title: Single-neuron representation of written words in human medial temporal lobe

Authors: *B. SAMIMIZAD[1], T. P. REBER[2], R. SURGES[1], V. BORGER[3], F. MORMANN[1]; [1]Univ. Hosp. Bonn, Bonn, Germany; [2]Psychology, UniDistance Suisse, Brig, Switzerland; [3]Dept. of Neurosurgery, Univ. Hosp. Bonn, Bonn, Germany

Abstract: Concept cells in the human medial temporal lobe (MTL) selectively respond to specific concepts, yet their semantic tuning and responsiveness to various properties of written words remain poorly investigated.
Here, we recorded neuronal activity from approximately 4,000 single neurons in 27 patients across 45 sessions in the hippocampus (HP), entorhinal cortex (EC), parahippocampal cortex (PHC), and amygdala (Amy) of human subjects undergoing intracranial epilepsy monitoring. Participants viewed word stimuli selected to orthogonalize four semantic and emotional dimensions—valence, arousal, concreteness, and word frequency. We analyzed responsive neurons to examine how specific word attributes modulate MTL activity.
Analyses revealed that hippocampal neurons were robustly modulated by word concreteness, exhibiting increased response probabilities, shorter response latencies, and stronger firing-rate modulation for concrete compared to abstract words. Notably, a significant number of hippocampal units also responded selectively to abstract words, suggesting dual engagement with both concrete and abstract semantic processing. In contrast, neuronal activity in the EC was significantly influenced by word arousal, as demonstrated using a generalized linear mixed-effects model, showing increased response probability and firing-rate modulation associated with higher-arousal stimuli. Additionally, a lateralization effect was observed, with a larger number of responsive units recorded in the left compared to the right hemisphere, predominantly driven by hippocampal neurons. Furthermore, we identified a subset of neurons responding to multiple words. These non-selective neurons tended to respond to semantically related stimuli significantly more often in HP and EC, but not in PHC, with Amy showing mixed evidence for semantic tuning.
Our findings clarify the distinct roles of hippocampal and entorhinal neurons in the semantic and emotional processing of language. These data underscore the nuanced specialization within human MTL subregions, highlighting the hippocampus's critical role in processing concreteness, semantic associations, and lateralized language representation, and the EC's involvement in arousal-driven modulation during word processing.

Disclosures: B. Samimizad: None. **T.P. Reber:** None. **R. Surges:** None. **V. Borger:** None. **F. Mormann:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

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Support: Volkswagen Foundation (86 506)
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German Research Council (MO930/4-2, MO930/15-1, SPP 2205, SPP 2411, SFB 1089)
NRW Network Grant (iBehave)

Title: Neuronal building blocks of episodic memory in the human medial temporal lobe

Authors: *S. MACKAY[1], M. BAUSCH[2], M. S. KEHL[3], T. P. REBER[4], F. MORMANN[5]; [1]Epileptology, Univ. of Bonn Med. Ctr., Bonn, Germany; [2]Epileptology, Univ. Hosp. Bonn, Bonn, Germany; [3]Exptl. Psychology, Univ. of Bonn / Univ. of Oxford, Bonn, Germany; [4]Psychology, UniDistance Suisse, Brig, Switzerland; [5]Univ. Clin. of Bonn, Bonn, Germany

Abstract: Episodic memory formation depends on the brain's ability to bind the 'what', 'where', and 'when' of experience into a coherent representation. However, the single-neuron mechanisms that support this process in the human medial temporal lobe (MTL) remain poorly understood. In a series of intracranial recordings from neurosurgical patients performing associative memory tasks, we identified two functionally specialized cell populations: concept cells in the hippocampus, entorhinal cortex, and amygdala that respond selectively to specific items, and location-selective neurons in the parahippocampal cortex (PHC). Both groups showed significantly more pronounced increases in firing rates during trials that were later successfully remembered, indicating their involvement in long-term memory encoding. These findings are in line with theories of hippocampal indexing, in which distinct neuronal subpopulations act as pointers linking neocortical content representations with contextual features.To probe the causal role of concept cells in memory formation, we employed low-dose propofol sedation during a similar associative memory task. This intervention led to a dissociation between intact perceptual processing and impaired associative memory. At the neural level, propofol generally altered the activity of concept neurons by attenuating their response magnitude and delaying peak response latencies. We investigated the extent of these effects in three MTL regions (hippocampus, amygdala and PHC) under different memory conditions and found: (1) stronger delays of hippocampal responses during disrupted source-memory encoding and (2) stronger attenuation of hippocampal and amygdala responses during disrupted item-memory encoding. These effects were absent in the PHC, suggesting regional specificity of memory-related coding.Together, our data reveal how distinct MTL neuron classes contribute to the encoding of content-context associations, and establish a causal link between concept cell activity and the formation of declarative memory traces in the human brain.

Disclosures: S. Mackay: None. **M. Bausch:** None. **M.S. Kehl:** None. **T.P. Reber:** None. **F. Mormann:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

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Program #/Poster #: PSTR412.17/NN12

Topic: I.07. Long-Term Memory

Title: Sequential coupling of sleep oscillations enables concept neuron reactivation and supports information flow across the human hippocampal-cortical circuit.

Authors: *F. SCHWIMMBECK[1,2,3], J. NIEDIEK[4], E. TRINKA[5,2], T. SCHREINER[3], F. MORMANN[6];
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Abstract: Effective memory consolidation during sleep is thought to depend on the transfer of reactivated memory traces from the hippocampus to the cortex. However, the mechanisms supporting this essential dialogue remain poorly understood.
To address this, we recorded single-neuron activity (N=1097) in the medial temporal lobe (MTL) of 10 epilepsy patients across 21 nights of sleep, after a memory task featuring concept neuron-linked stimuli.
During non-rapid eye movement (NREM) sleep, sharp wave-ripple (SWR)-locked neuronal firing revealed a directed flow of information from the hippocampus to cortical targets. Notably, within SWR-driven activity, experience-dependent concept neurons exhibited elevated co-activation both locally and across MTL regions, indicating selective reactivation of behaviorally relevant information. Cross-regional co-activation of concept cells was markedly enhanced when hippocampal SWRs coincided with cortical slow oscillation-spindle complexes, suggesting an active role of the neocortex in shaping interregional communication.
These findings provide evidence that SWRs in humans selectively reactivate experience-related neural ensembles across the hippocampal-cortical network, while synergistic interactions with cortical slow oscillation-spindle sequences might facilitate effective memory consolidation.

Disclosures: F. Schwimmbeck: None. **J. Niediek:** None. **E. Trinka:** None. **T. Schreiner:** None. **F. Mormann:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

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Program #/Poster #: PSTR412.18/OO1

Topic: I.07. Long-Term Memory

Support: BMBF; 01GQ1705A and 01GQ2402A
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Studienstiftung des deutschen Volkes
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DFG; Projektnummer 447634521
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Title: A dataset of human single-neuron and intracranial EEG recordings during a spatial memory task

Authors: *T. A. GUTH[1], A. BRANDT[2], P. C. REINACHER[3], M. F. KHAZALI[2], A. SCHULZE-BONHAGE[2], J. JACOBS[4], L. KUNZ[1];
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Abstract: Spatial memory is a fundamental cognitive function that enables humans to remember locations, navigate through environments, and interact with their surroundings. While extensively studied in rodents, opportunities for investigating the neural mechanisms underlying spatial memory in humans remain limited. Here, we present a dataset of intracranial recordings from the medial temporal lobe of 18 epilepsy patients who underwent presurgical evaluation with hybrid depth electrodes. Participants completed 27 sessions of a spatial memory task in which they navigated a virtual environment to encode object locations, followed by a distractor task and a cued recall phase. The dataset is formatted according to the Neurodata Without Borders standard and includes single-neuron spike times and waveforms, local field potentials, intracranial EEG recordings, electrode localizations, behavioral data, and task-related information. For validation, we provide assessments of recording quality, behavioral performance, spike-field relationships, and spatial tuning of single neurons. This large dataset of intracranial recordings offers an accessible resource for investigating the neural correlates of human spatial memory at high spatiotemporal resolution.

Disclosures: T.A. Guth: None. **A. Brandt:** None. **P.C. Reinacher:** None. **M.F. Khazali:** None. **A. Schulze-Bonhage:** None. **J. Jacobs:** None. **L. Kunz:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

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Program #/Poster #: PSTR412.19/OO2

Topic: I.07. Long-Term Memory

Support: BMBF; 01GQ2402A

Title: Single-neurons in the human parahippocampal cortex represent spatial distances

Authors: *D. WOLF[1], V. BORGER[2], R. SURGES[1], L. KUNZ[1], F. MORMANN[1];
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Abstract: Flexible behavior in dynamic environments relies on an internal model of the world—a cognitive map. Spatial distance to goals or conspecifics is a critical variable in planning behaviors such as goal-directed approach, threat avoidance, and social interaction. In non-human animals, specific cell types—such as goal-vector and vector-boundary cells—encode egocentric or allocentric distances to relevant objects. However, how spatial distance is represented in the human medial temporal lobe (MTL) remains poorly understood.
To address this, we recorded single-neuron activity from the MTL of 13 epilepsy patients undergoing intracranial monitoring. Across 18 recording sessions, we recorded 890 single and multi-units. During two experimental conditions, patients either viewed an individual standing at one of four distinct distances for two seconds (static condition) or fixated on an individual moving continuously toward or away at a constant speed (dynamic condition).
We found that neurons in the parahippocampal cortex encoded the distance to another person under both static and dynamic conditions. These responses were stable and spanned the full range of presented distances. Our preliminary findings suggest that the human MTL contains a cellular representation of spatial distance to others. More broadly, these results provide new insights into the neural basis of spatial coding and its role in social perception in humans.

Disclosures: D. Wolf: None. **V. Borger:** None. **R. Surges:** None. **L. Kunz:** None. **F. Mormann:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.20/OO3

Topic: I.07. Long-Term Memory

Support: SNSF 204651

Title: Automated detection separates physiological ripples during a verbal working memory task from epileptic high frequency oscillations

Authors: V. DIMAKOPOULOS[1], F. COSTA[1], J. TEURLINGS[2], L. IMBACH[3], D. LEDERGERBER[4], ***J. SARNTHEIN**[5];
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Abstract: Intracranial EEG from the medial temporal lobe (MTL) of patients with epilepsy shows both physiological ripples and epileptic high frequency oscillations (HFOs). The distinction between the two types of events is a matter of debate. We recorded intracranial EEG

in bilateral MTL from 14 patients while they performed a verbal working memory task where letter strings of set size 4, 6, or 8 were presented. Letters were selected from 15 consonants and shown during the encoding stage (duration 2 s), followed by the maintenance stage (3 s) and visual presentation of a probe letter, which initiated the retrieval stage [*eLife*, 2022]. Using two fully automated detectors, we identified physiological ripples and epileptic HFOs [*Brain*, 2025]. One MTL was subsequently resected. Postsurgical seizure outcome was dichotomized in seizure free (ILAE1) or seizure recurrence (ILAE2-6). During the performance of a trial, there was a significant clustering of ripples during encoding of memory items and during memory retrieval ($p < .05$), speaking for the physiological ripples as markers of memory function. The rate of epileptic HFOs was not modulated by any of the task stages ($p > .05$). Across all patients, the number of physiological ripples (29693) was higher than the number of epileptic HFOs (8276). The distributions of amplitude and center frequency largely overlapped between ripples and HFOs. Nevertheless, only 5 HFOs per patient (1%) coincided with a physiological ripple. There was no evidence that the ripple rate would be higher in the left than the right MTL of the patients ($p > .05$). Across the 9 patients who achieved postsurgical seizure freedom (ILAE1), the median HFO rate during the trial (.06 IQR [.04 .11] Hz) was higher in the epileptogenic MTL compared to the healthy MTL (.02 IQR [.01 .03] Hz), speaking for HFOs as markers for epileptic tissue. We demonstrate a well-defined setup with automated detection that separates physiological ripples, which indicate memory function, from epileptic HFOs, which indicate epileptic tissue.

Disclosures: V. Dimakopoulos: None. **F. Costa:** None. **J. Teurlings:** None. **L. Imbach:** None. **D. Ledergerber:** None. **J. Sarnthein:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

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Program #/Poster #: PSTR412.21/OO4

Topic: I.07. Long-Term Memory

Support: Canada Research Chairs Program (950–233046)
Canadian Hearing Services Global Partnerships for Research & Innovation
NSERC Postgraduate Scholarships-Doctoral Fellowship

Title: Hippocampal clues to the interconnection between episodic memory deficits and hearing impairment in chemotherapy-treated cancer survivors

Authors: I. X. MACPHEE[1], K. D. MUNELITH-SOUKSANH[3], M. BRADLEY-GARCIA[4], J. A. ANDERSON[2], *M. J. SEKERES[4];

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Abstract: Background: While life-saving, chemotherapy can lead to neurotoxic side effects (chemofog) including hearing loss and cognitive decline. The hippocampus, critical for episodic memory and increasingly recognized for its role in auditory processing, may serve as a key neural structure mediating these effects. However, the impact of chemotherapy and hearing loss on distinct hippocampal subfields remains poorly understood. To address this, we applied HippUnfold, a machine learning-based segmentation tool, to examine hippocampal subregion volumes in breast cancer survivors. **Methods:** Breast cancer survivors and age-matched healthy controls underwent 3T MRI scanning, and were assessed with hearing (audiometry, QuickSIN), and cognitive assessments (Shipley-2, verbal paired associates, and film clip based event memory tasks). **Results:** Total hippocampal and subfield volumes were lower in the treatment group, and high-frequency hearing loss was present in 67% of the treatment subset. Exploratory multiple factor analysis revealed a first dimension with 49.8% of the variance explained (VE), associated with education, age, and hearing, and trending with right dentate gyrus volume. In the second dimension (23.8% VE), cognitive measures and age loaded with right CA3 volume. The third dimension (10.1% VE) was driven by cognition, hearing, and education, alongside left CA1 and CA3 volumes. Deficits in episodic elements of complex event memory was observed in cancer survivors, in addition to higher reported cognitive workload during memory processing tasks. **Conclusions:** Hippocampal subregions may be differentially vulnerable to the effects of systemic chemotherapy treatments, contributing to memory impairments observed in cancer survivors. Chemotherapy-induced ototoxicity and associated hearing impairment likely exacerbates these deficits, increasing cognitive load and impacting quality of life. These findings identify novel avenues for therapeutic interventions that address both neurocognitive and sensory health in cancer survivorship.

Disclosures: I.X. MacPhee: None. **M. Bradley-Garcia:** None. **J.A. Anderson:** None. **M.J. Sekeres:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

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Program #/Poster #: PSTR412.22/OO5

Topic: I.07. Long-Term Memory

Support: 24KKSGR017

Title: Narrative Order Remaps Time Coding Subspaces While Preserving Geometric Structure

Authors: *X. ZHU[1], Z. JIN[1], H. JIANG[2], K. PAN[2], S. KWOK[1];
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Abstract: Objective Episodic memory relies on the brain's ability to represent the temporal structure of continuous experience. However, how disruption in the natural flow of events affects temporal encoding and retrieval remains unclear. Here, we examine how reversing narrative flow affects the neural representation of time during memory formation and recall. **Methods** Intracranial EEG (iEEG) was recorded from nine epilepsy patients performing a temporal order judgment (TOJ) task. On each trial, participants viewed a 4~6 s video clip and later selected the chronologically earlier of two extracted frames. Each video was shown twice -- once in natural and once in reversed order. Broadband (1-150 Hz) activity across whole-brain electrodes was analyzed using representation similarity analyses (RSA), decoding, and neural subspace geometry methods. **Results** Behaviorally, disrupting event flow increased reaction time without impairing accuracy. Neural patterns during encoding tracked temporal distances between video segments, with increased similarity in the Reversed condition. During retrieval, post-stimulus activity (1~1.5 s) reflected the temporal positions of the TOJ target frames. Time position could be decoded from whole-brain signals (20.46 ± 4.99 % vs. chance = 12.5 %) irrespective of narrative direction. Subspace analysis revealed that while both conditions maintained similar geometry of time codes, they occupied orthogonal subspaces. UMAP embedding showed trial-specific retrieval trajectories and replay-like dynamics, with predicted temporal position progressively rising. In the Reversed condition, retrieved time estimates deviated significantly from actual TOJ targets ~~2 s post-onset. **Conclusion** Our findings reveal that disruption of narrative flow reorganizes time codes into orthogonal neural subspaces while preserving their geometric structure. This dual mechanism highlights the flexibility of temporal coding and shows how subjective time emerges from structural scaffolding of experience.

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Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

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Program #/Poster #: PSTR412.23/OO6

Topic: I.07. Long-Term Memory

Support: Schwartz Discovery Fellowship to OM
Meliora Fellowship to OM

Title: Expertise facilitates event perception and memory recall for naturalistic narratives

Authors: *O. MEULBROEK[1], C. R. IORDAN[2];
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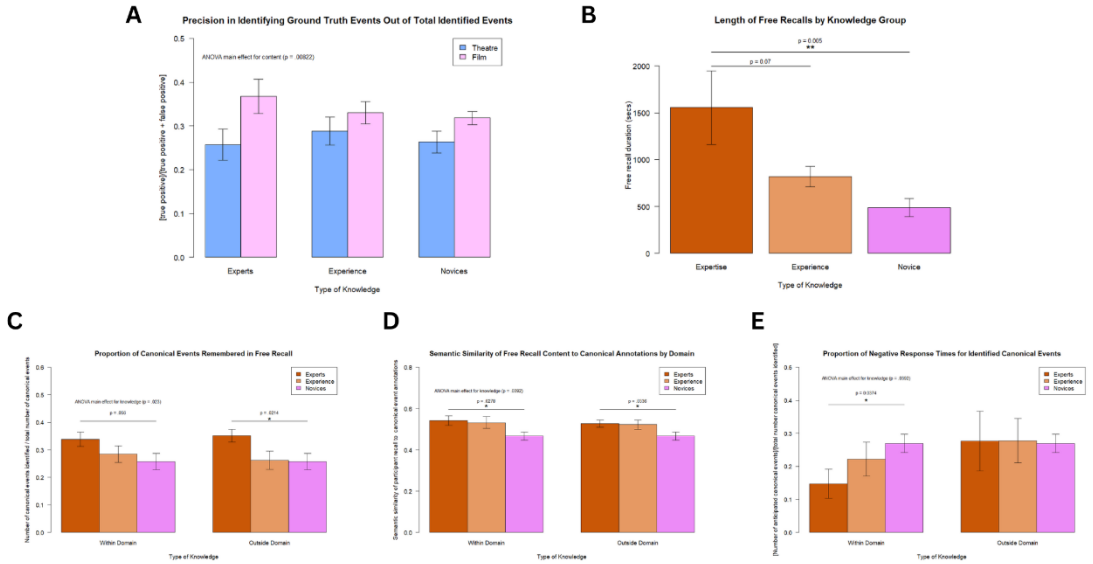
Abstract: Our prior knowledge influences the way we experience and remember the world. Expertise enhances perception and segmentation of structured activities into individual events (Newberry et al. 2021). However, it remains unclear how expertise affects perception and

memory for unstructured continuous experiences, such as naturalistic narratives. Additionally, it is unknown whether experience with a particular domain (e.g., theatre production involvement) influences memory similarly to expertise (e.g., B.A. in Theatre). We hypothesize that experience with and expertise in a domain (film/theatre) will facilitate memory encoding and recall for naturalistic narratives for that particular domain, with expertise having a stronger influence on memory than experience. We recruited three cohorts (experts, experienced, novices) and showed them six 10-min clips-3 theatre, 3 film. During viewing, they identified event boundaries by keypress. Afterwards, they freely recalled the clips' plots. We found that content type influenced memory: regardless of knowledge, participants had higher precision, faster reaction times, and higher likelihood to recall canonical events in film compared to theatre (ANOVA p=.008, p=.043; p=.037). As knowledge about their preferred domain increased, the length of participants' recalls increased (ANOVA p=.005) and the semantic content of recalls aligned more with canonical descriptions of clips, both within and outside the preferred domain (proportion events remembered, ANOVA p=.023; semantic similarity to canonical model, ANOVA p=.039). Surprisingly, we also found that experts anticipated fewer canonical boundaries in their preferred domain compared to novices (proportion negative response times for canonical boundaries, p=.037), despite the same base rate of identifying canonical events (p=.342). Together, our results suggest that domain expertise facilitates perception and memory for naturalistic narratives within and outside of the preferred domain, and that expertise may affect event segmentation in a domain-specific manner.



Disclosures: O. Meulbroek: None. **C.R. Iordan:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

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Program #/Poster #: PSTR412.24/OO7

Topic: I.07. Long-Term Memory

Support: 32441110
32330039

Title: Dissociated neural coding of global and local positions in sequence memory for naturalistic stimuli

Authors: ***X. PENG**, G. XUE;
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Abstract: Real life experience unfolds continuously across multiple timescales, yet how the brain represents these hierarchical event sequences remains poorly understood. In this study, we investigated the neural coding of hierarchical positions in naturalistic sequence memory. During magnetoencephalography recordings, participants in Experiment 1 (N = 26, 7 male; M = 21.69 years) viewed video clips with a hierarchical structure and later recalled their serial position. Experiment 2 (N = 33, 7 male; M = 21.88 years) served as a behavioral control, using sequences lacking the hierarchical structure. Behavioral results revealed more transposition errors in Experiment 1 for items that shared the same global or local position, compared to the control. Classification analyses showed that both global and local position representations during encoding predicted subsequent memory of serial order. Local positions were better decoded in the original sensor space, whereas global positions exhibited greater pattern separability in neural subspaces, indicating better preservation when projected from high- to low-dimensional neural spaces. Both classification and subspace analyses revealed separable neural representations of global and local positions. Compared to brain regions encoding local positions, those encoding global positions displayed longer neural timescales during both non-task and video-watching periods, along with less sensitivity to local event transitions. These findings motivate a hierarchical factorization framework for sequence representations, suggesting orderly correspondence between levels of position coding and event processing, embedded within inherent hierarchical neural architecture.

Disclosures: X. Peng: None. **G. Xue:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

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Program #/Poster #: PSTR412.25/OO8

Topic: I.07. Long-Term Memory

Support: NSFC 62061136001/DFG TRR169

Title: Modulation of Human Spatial-temporal Memory by Deep Brain Stimulation

Authors: *Y. LI[1], G. XUE[2];
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Abstract: Modulation of human spatiotemporal memory by deep brain stimulation
Authors Yan Li[1], Gui Xue[1] [1]State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing, China **Disclosures** Yan Li: None. Gui Xue: None. **Abstract** Epilepsy patients experience memory malfunctioning in varying degree. Deep brain stimulation (DBS), a neuromodulation approach for seizure control, has also been studied for its potential to restore or enhance memory functions. However, the optimal target and parameters for stimulation to improve memory remain undefined, and the mechanisms by which deep brain stimulation modulates memory-related neurophysiological processes are not well understood. Twenty-seven medically refractory epilepsy patients (*M* = 24.03y, *s.d.* = 8.36y, 9 female) who were implanted with SEEG depth electrodes were recruited in Xuanwu hospital, China. Participants were required to complete a modified Corsi test. Stimulation parameters were set to a current density of 0.5mA, with a pulse width of 90μs delivered at either 5 Hz or 50 Hz, while sham stimulation maintained identical electrode placement without current delivery. Stimulation targeted either the hippocampal proper or its adjacent white matter tracts, with electrode placement located within or outside the seizure onset zone (SOZ). We found that epilepsy patients derived the greatest benefit when stimulation was applied outside the SOZ. Besides, improved memory performance was observed when stimulation was delivered at 50 Hz targeting hippocampal white matter, whereas impaired performance was associated with 5 Hz stimulation directed toward the hippocampal proper. We also identified both regional and global biomarkers that could predict behavioral outcomes induced by stimulation. Stimulation targeting regions within task-relevant networks effectively modulated memory-related processes. Moreover, 50 Hz stimulation directed at white matter facilitated transitions in brain states toward to those optimized for memory processing, thereby improving memory performance. Understanding how DBS modified localized activity and brain states to influence memory might inform future therapeutic strategies.

Disclosures: Y. Li: None. **G. Xue:** None.

Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR412.26/OO9

Topic: I.07. Long-Term Memory

Support: NIH-NINDS R01-NS089729
NIH-NINDS F31NS126016

Title: Ca3 and dentate gyrus adaptively inverse the behaviorally-relevant dimensions of visual similarity

Authors: *S. MIRJALILI[1], W. GUO[2], D. GRAETZ[2], E. WANG[2], U. MAYR[2], B. A. KUHL[2]; [2]Psychology, [1]Univ. of Oregon, Eugene, OR

Abstract: The hippocampus is thought to minimize interference among similar memories by orthogonalizing neural codes (Yassa et al., 2011). However, orthogonalization fails to account for recent evidence of more dynamic and reactive processes in the hippocampus. In particular, at least in certain contexts, similarity has been shown to trigger an active 'repulsion' of hippocampal representations (Favilla et al., 2016; Wanjia et al., 2021; Hulbert et al., 2015). To the extent that repulsion is an adaptive reaction to similarity, an interesting possibility is that repulsion specifically occurs along stimulus dimensions that are currently relevant to behavior. Here, we tested this idea by identifying distinct dimensions of stimulus similarity and testing whether hippocampal repulsion preferentially occurred along dimensions that produced memory interference. To identify relevant dimensions of similarity, we generated a set of stimuli (images) spanning two visual categories and then computed the pairwise similarity of these images using a combination of neural networks, artificial intelligence, and human perceptual and memory decisions. By applying PCA to resulting similarity matrices, we identified two dimensions that explained over half of the variance in similarity. Critically, these two dimensions powerfully (and selectively) predicted interference-related memory errors in an independent data set. Using high-resolution fMRI, we then tested whether the hippocampus was sensitive to these two dimensions of similarity. Strikingly, subfields CA3 and dentate gyrus (CA3/DG) robustly inverted similarity along both dimensions—a phenomenon consistent with repulsion, but inconsistent with orthogonalization. However, repulsion occurred, and disappeared, for each dimension at different stages of learning. Inspired by these fMRI data, we then established—and replicated—that these two dimensions are also differentially related to behavior at different stages of learning, strongly paralleling the dynamic repulsion effects we observed in CA3/DG. Collectively, these findings reveal that CA3/DG adaptively transforms behaviorally-relevant dimensions of visual similarity in service of reducing memory interference.

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Poster

PSTR412: Human Long Term Memory: Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: I.07. Long-Term Memory

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Title: Semantic boundaries facilitate temporal memory organization through cortico-hippocampal event segmentation

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Abstract: Humans can efficiently remember diverse and continuous experiences by segmenting episodic memories into meaningful events. Event boundaries serve as points of segmentation and have been reported to enhance memory within an event while disrupting memory across events. Given the centrality of semantic information in human cognition, we investigated how semantic boundaries impact the segmentation and temporal organization of episodic memory and its cortico-hippocampal neural correlates. 94 participants encoded ten-word sequences during fMRI scanning. In no-boundary trials, all words belonged to a single semantic cluster defined by Word2Vec embeddings, whereas boundary trials included a cluster shift midway through the sequence. In a subsequent temporal order judgment task, semantic boundaries significantly improved memory accuracy, especially for across-boundary judgments. A temporal distance effect on task performance was observed only for within-boundary judgments, suggesting that semantic boundaries promote hierarchical event structuring. At the neural level, semantic boundaries elicited greater activation in cortical regions associated with event segmentation, particularly in the lingual gyrus and high-level visual areas. Temporal dynamics of neural pattern similarity revealed a shift in hippocampal representation across semantic boundaries, and increased similarities among post-boundary items. Similar representational shifts were observed in the lingual gyrus, parahippocampal cortex, and orbitofrontal cortex, suggesting coordinated cortico-hippocampal segmentation during memory encoding with semantic boundaries. Our findings demonstrate that semantic boundaries shape how we encode temporally sequenced events by triggering coordinated cortico-hippocampal activation and modulating the similarity of item representations across those boundaries.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

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Topic: I.08. Learning and Memory

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Sloan Fellowship

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Title: Circuit mechanisms of hippocampal remapping and drift during rapid generalization

Authors: *H. CHANG[1], W. TANG[1], C. LIU[3], S. PEREZ HERNANDEZ[1], W. ZHENG[4], J. PARK[1], A. FERNANDEZ-RUIZ[1], A. OLIVA[2];
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Abstract: Acquiring new knowledge and generalizing from experience is critical for intelligent behavior. Initial learning relies on the hippocampus, which can generate stable representations to discriminate across episodic memories. Paradoxically, its neural representations are highly unstable, as hippocampal neural representations remap across environments and exhibit continuous drift over time. Such instability raises the question of how hippocampal neurons can encode reliable memory representation of different experiences over time. Crucially, our analyses revealed that while place cell remapping and drift appear to be random at the single cell level, they are highly structure processed at the population level, characterize by different stereotypical dynamics of the hippocampal neural manifold. hypothesized that these two dynamic processes, spatial remapping and temporal drift, are driven by distinct underlying circuit mechanisms. Using large-scale recordings from the hippocampus computational modeling, and optogenetic manipulations , we trained mice mice to perform a delayed spatial alternation task across different mazes. We found that CA1 place cell's remapping across environments is facilitated by feedforward inputs from its upstream regions, including CA3 and medial entorhinal cortex (MEC). Both a CA3-CA1 network model and experimental results demonstrated that, despite global CA1 remapping, CA1 cells within the same CA3-CA1 cell assembly show correlated remapping and CA3-CA1 communication subspaces are preserved across mazes. Disrupting either CA3 or MEC inputs, induced global remapping in CA1—manifested as a significant rotation of the neural manifold—but notably did not affect the ongoing temporal drift of neuronal activity. Conversely, disturbing local parvalbumin inhibition interfered with temporal drift, resulting in stabilized spatial representations and impaired memory performance without causing remapping. These results show that spatial remapping and temporal drift are governed by distinct and separable circuit mechanisms: while hippocampal remapping supports memory discrimination across environments, hippocampal drift is essential to provide an independent coding axis for episodic time.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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R00MH120343
R00MH122582
New Frontiers Grant
Sloan Fellowship
Whitehall Research Grant
MIND Prize from the PSF

Title: Decoding group rodent social interactions with an AI-enabled responsive multi-modal wireless neurologger

Authors: ***Z. ZHAO**[1], H. CHANG[2], J. PARK[2], C. LIU[2], P. PAUDEL[2], A. FERNANDEZ-RUIZ[2], A. OLIVA[2];
[2]Neurobio. and Behavior, [1]Cornell Univ., Ithaca, NY

Abstract: Understanding the neural mechanisms that generate behavior requires the ability to record and manipulate brain activity under rich, unrestrained, and close to natural conditions. However, traditional methods often restrict animal movements, limit group interactions, and fail to scale to large, complex environments where animal's live in. To address these challenges, we developed a wireless, responsive neurologger with integrated multi-modal behavioral monitoring. The platform incorporates custom flexible neural probes, neural amplifier, optogenetic driver, inertial measurement unit, ultrasonic microphone, and miniature head-mounted camera—all within a <1.5 g device that imposes minimal constraints on animal movement. Using this system, we were able to perform experiments that were previously infeasible. We recorded neural and behavioral activity from groups of mice in both standard lab environments and large outdoor enclosures, capturing interactions among up to seven animals simultaneously. Integrated sensors allowed real-time detection of ultrasonic vocalizations and eye movements during social interactions. Implanted mice exhibited no significant reduction in exploration or social interactions compared to unimplanted controls. In addition, onboard data-driven signal-processing pipelines were implemented with tinyML for real-time detection and classification of both behavioral and neural patterns. These capabilities enabled closed-loop optogenetic manipulations in awake, freely moving animals based on the detection of specific behavioral "syllables" or neural activity events. We found that neural assemblies across hippocampal and cortical regions exhibit mixed selectivity, encoding multiple behavioral and contextual variables. Our multi-modal recordings allowed us to describe, for the first time, pupil dynamics, vocalizations, and neural activity during mice group social interactions. Together, this platform provides a powerful tool for mechanistic investigations of brain activity during complex, naturalistic behaviors.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Title: A hippocampal population code for rapid generalization

Authors: *W. TANG, H. CHANG, C. LIU, S. PEREZ HERNANDEZ, W. Y. ZHENG, J. PARK, A. OLIVA, A. FERNANDEZ-RUIZ;
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Abstract: Humans and other animals can rapidly adapt their behaviors in response to changes in the environment. This rapid learning depends on their capacity to generalize from previous experiences and apply learned knowledge to novel contexts. Traditional models, including the standard theory of complementary learning systems (*McClelland et al., 1995*), assert that while the hippocampus effectively learns the details of present experiences, the neocortex is responsible for generalization by gradually forming structured knowledge from past experiences. However, such a gradual process fails to explain how animals can quickly generalize from limited experiences. In the present study, we recorded large neuronal ensembles in the dorsal area CA1 of the hippocampus (300 neurons simultaneously) as mice generalized learned knowledge in a hippocampal-dependent memory task across three distinct environments. We found that, although individual CA1 neuron activity showed random changes over time and across different environments, the neuronal population maintained a distinct geometric organization of memory information. First, different aspects of experience were encoded independently in a disentangled manner, whereby changes in one aspect of the hippocampal representation (e.g., spatial dimension) did not affect the encoding of other attributes (e.g., temporal dimension). Second, this disentangled representation developed in tandem with the learning process and readily generalized to new environments, despite a complete reorganization of single-cell responses. Thus, these two critical geometric properties could underlie the rapid generalization of prior knowledge for new tasks. Together, these findings suggest that the hippocampus performs crucial computations to facilitate rapid generalization and flexible learning.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Title: Naturalistic foraging reveals environment-specific behavioral motifs and hippocampal dynamics in freely moving rats

Authors: *C. LIU[1], J. SHEN[2], Z. ZHAO[3], M. AURELIO[3], E. CARBAJAL LEON[3], G. TSIMIS[2], Z. SUHAIL[2], A. OLIVA[3], A. FERNANDEZ-RUIZ[3];
[1]Cornell Univ. Neurobio. and Behavior, Ithaca, NY; [3]Neurobio. and Behavior, [2]Cornell Univ., Ithaca, NY

Abstract: Traditional controlled laboratory studies have enabled us to understand many principles of hippocampal function. However, the brain evolved to help animals survive in dynamic, complex environments, raising the question of whether and how these principles operate in the real world. To address this, we recorded hippocampal CA1 activity while rats foraged freely in two large arenas that differed only in their context: an indoor enclosure and an outdoor natural field site of equal footprint but with real-world sensory complexity. We conducted recordings using silicon probes and custom wireless electrophysiology loggers featuring an onboard IMU, and an overhead camera for tracking behavior. We first set out to characterize differences in locomotion and foraging patterns in the two contexts. To this end, we developed a new pipeline that fuses video kinematics with IMU signals to segment continuous movement into recurring behavioral syllables. We found prominent differences in navigation and foraging strategies between the two environments. Indoors, rats sampled the full arena scanning the environment homogeneously with long, continuous trajectories. In contrast, outdoors, rats covered a smaller fraction of the arena, repeatedly traversing a small set of preferred corridors flanked by increased local sensory sampling. Notably, a novel hopping behavior emerged outdoors when animals bridge exploited and unexplored patches, suggesting a more deliberative foraging behavior. Hippocampal signatures, including place cells, theta sequences, and replays, were expressed reliably in the outdoor arena, underscoring the ecological validity of classic findings. Context-dependent shifts in these hippocampal signatures could be explained by the locomotion and foraging strategy differences in these two environments. Our results suggest that

naturalistic contexts reshape the micro-structure of behavior, and the hippocampal code is reorganized and closely tied to naturalistic behavioral motifs. We propose that linking fine-grained ethology with large-scale neural recordings is essential for understanding brain principles that support behaviors in the real world.

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Poster

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Location: SDCC Halls B-H

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Topic: I.08. Learning and Memory

Support: NIH R00MH122582
Cornell Univ. New Frontiers Grant (AO)

Title: Hippocampus Constructs and Maintains Cognitive Maps of Social Territories in a Natural Environment

Authors: *P. PAUDEL, Z. ZHAO, R. JOE, R. NIRAULA, X. MEI, A. YU, E. ZHAO, W. ZHENG, A. FERNANDEZ-RUIZ, A. OLIVA GONZÁLEZ;
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Abstract: The neural basis of rodent's spatial and social behaviors is typically studied independently in reduced lab environments that fail to capture the complexity of their natural behavior. In nature, mice establish and defend territories while displaying various affiliative and aggressive social behaviors, yet how the brain represents and uses environmental and social cues to guide such territorial behavior remains unknown. We investigated how hippocampal activity patterns important for learning and memory are modulated by spatial and social organization in male mice living in an outdoor field enclosure (~2000× larger than typical lab mazes). Adult male and female mice were released into this semi-natural environment containing monopolizable resource zones. We recorded hippocampal neural activity and animal behavior during territorial establishment, social interactions, foraging and patrolling behaviors for 2 weeks. Our data suggests that mouse social interactions are strongly influenced by spatial context (territories). Male mice establish territories, actively survey their social environment, and adapt to notable alterations within it. More importantly, male-male interactions trigger patrolling behavior once territories are established, but not during early phases when territories have not yet been established. Hippocampal place cell activity showed context-dependent modulation, with distinct firing patterns associated with territorial boundaries and social encounter zones. Cell assemblies encoded conjunctively spatial and social variables. These findings suggest that the hippocampus constructs and maintains a cognitive map of the territories that integrate spatial and

social information in nature. The interdependence of spatial representation and social context in hippocampal coding may be fundamental to understanding how the brain guides territorial and other types of natural behaviors. These results highlight the importance of studying the neural mechanisms that generate behavior in ecologically relevant contexts with the goal of understanding brain function.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Title: Systems Memory Consolidation revisited: Closed-loop optogenetic manipulations reveal new roles of bidirectional Hippocampal-Cortical in memory consolidation and recall

Authors: *X. MEI, R. E. HARVEY, C. LIU, Z. ZHAO, W. CHEN, K. GUPTA, E. CARBAJAL LEON, T. NETZER, A. OLIVA, A. FERNANDEZ-RUIZ;
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Abstract: The mechanism of memory consolidation, the process of transforming newly encoded representations into long-term memories, is not yet fully understood. The classic two-stage theory proposes that memory is initially encoded in the hippocampus (HPC) during exploratory behaviors and subsequently transferred to the neocortex for long-term storage. In later rest periods, sharp-wave ripples (SWRs) facilitate initial consolidation through hippocampal reactivation, as well as the transfer of memory traces from HPC to prefrontal cortex (PFC). While the role of cortical inputs in shaping hippocampal replay dynamics remains unclear, cortical slow oscillations of UP (depolarized) and DOWN (hyperpolarized) states are temporally coupled with SWRs and often tied to memory consolidation. In the current study, we used dual-site, high-density neural recordings in rats with event-specific closed-loop optogenetic silencing in PFC to dissect the role of bidirectional communications between HPC and PFC. Rats were

trained to learn different reward configurations in the "cheeseboard" maze every day. Theta oscillation-dependent PFC silencing during memory acquisition did not affect learning. In contrast, the same manipulation applied during testing, 22 hours later, severely impaired performance, underscoring the importance of top-down cortical inputs in memory recall. During the post-task sleep, bilateral PFC silencing triggered by hippocampal ripples disrupted memory consolidation and impaired its recall 22 hours later. In contrast to traditional assumptions, we identified non-REM sleep cortical UP-states as the key phase for PFC-to-HPC feedback, as transiently silencing PFC at the UP-state onset immediately after learning also impaired consolidation and memory performance. Consistent with the behavioral outcomes, hippocampal replay was suppressed by closed-loop PFC silencing during SWRs and more strongly during cortical UP state but not with randomly timed manipulations. In summary, our findings suggest that PFC-HPC coordination is essential for memory recall despite not being necessary for initial acquisition in the same task. Furthermore, cortical feedback during UP-states in the non-REM sleep immediately following new learning is necessary for consolidation and hippocampal replay. These results highlight the essential contribution of PFC to memory consolidation much earlier than what is assumed by traditional models.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Title: Systems Memory Consolidation revisited: Prefrontal Cortex is necessary for hippocampal replay and memory consolidation immediately following new spatial learning

Authors: *R. E. HARVEY[1], C. LIU[2], Z. ZHAO[2], W. CHEN[1], X. MEI[1], K. GUPTA[1], T. NETZER[1], E. CARBAJAL LEON[1], A. OLIVA[1], A. FERNANDEZ-RUIZ[2];
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Abstract: Memory consolidation, the process of transitioning memories from initial encoding to long-term storage, remains a central focus in neuroscience. It is hypothesized that a dialogue between the hippocampus (HPC) and prefrontal cortex (PFC) is required, but how these structures interact to support memory consolidation remains poorly understood. The canonical model posits that memories are initially encoded in the hippocampus and then gradually transferred to the cortex for long-term storage. Sharp-wave ripples (SWRs) are thought to mediate initial consolidation in the hippocampus and subsequent HPC-to-PFC memory transfer. The cortex also sends direct and indirect projections to the HPC, but the role of cortical feedback in shaping hippocampal dynamics is unclear. Here, we combined high-density neural recordings in rats with event-specific closed-loop optogenetic silencing of PFC during post-learning sleep to dissect this HPC-to-PFC bidirectional interaction. We hypothesized that, in contrast to the traditional assumption of a sequential involvement of HPC first and PFC later in the memory consolidation process, PFC inputs may be instrumental already in the initial stages to select the appropriate memory traces to replay and consolidate in the hippocampus. We observed that, following novel spatial learning in a cheeseboard maze task, both HPC and PFC exhibited enhanced memory reactivation around SWRs. Closed-loop optogenetic silencing of PFC during sleep after learning disrupted memory recall the next day and, surprisingly, degraded hippocampal memory replay. These effects were stronger when silencing was targeted to the initial part of the non-REM sleep UP state in the PFC, compared to the later part or random timing. This result suggests that the initial UP state, when PFC is highly active, is a specific temporal window in which cortical top-down inputs are necessary for HPC replay. When rats learned different reward configurations in a single day, PFC silencing following learning of one configuration specifically disrupted its replay, while sparing replay of the other configuration. This suggests that the role of PFC inputs is to prioritize the appropriate memory trace to replay. By demonstrating that bidirectional HPC-PFC coordination is essential for both replay and behavioral outcomes, our work crucially extends the two-stage model of memory consolidation, highlighting the PFC as an active counterpart to the HPC in enabling memory persistence.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

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Sloan Fellowship
Whitehall Research Grant
MIND Prize

Title: Tracing Cognitive Maps: Long-Term Place Cell Dynamics via a Soft Neural Interface

Authors: *J. PARK[1], Z. ZHAO[2], L. KARABA[2], S. PEREZ HERNANDEZ[1], W. ZHENG[1], A. KWON[1], A. OLIVA[1], A. FERNANDEZ-RUIZ[2];
[2]Neurobio. and Behavior, [1]Cornell Univ., Ithaca, NY

Abstract: The neural mechanisms underlying the formation and long-term dynamics of cognitive maps remain elusive. Multiple studies have elucidated how the exploration of a novel environment leads to the formation of place cells, their coordination into synchronous cell assemblies and their subsequent consolidation through reactivation during sleep. However, studying this process with cellular resolution over extended timescales poses significant challenges. Long-term, multi-region recordings—particularly in the hippocampus (HPC) and prefrontal cortex (PFC)—are crucial for capturing how spatial codes emerge and evolve at both the population and single-cell levels. However, rigid silicon probe recordings cannot track the same cells across multiple days while calcium imaging lacks sufficient temporal resolution and multi-regional coverage. To overcome these limitations, we developed an ultrathin, high-density soft neural probe composed of a 3 µm-thick Parylene-C substrate with a multilayered Au/Pt/PEDOT thin-film electrode array. This design minimizes tissue response and enables chronic, large-scale recordings with 128-256 channels across multiple brain regions in freely behaving mice over several months. Using these probes to record from PFC and CA1 region of the HPC, we achieved stable single-unit recordings from large numbers of neurons for more than six months. Using this system, we trained rodents in a familiar environment and subsequently introduced them to a novel space. We found that the recorded neural population comprises two functional subgroups: stable cells, which maintained consistent place fields over time, and drifting cells, which exhibited variable spatial tuning. We also examined the formation, composition and evolution of cell assemblies and neural sequences, and their reactivation during sleep across multiple weeks. We hypothesize that stable cells act as anchoring nodes, rapidly forming connections that supported fast and coherent remapping of the cognitive map. Our findings can reveal the interplay between stability and plasticity for the development of cognitive maps . Importantly, this work can provide new insights into how enduring and adaptable neural codes support memory over extended timescales, including the transition between physiological and pathological conditions.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.09/PP1

Topic: I.08. Learning and Memory

Support: Brain Research Foundation BRFSG-2021-11
Norman H. Anderson

Title: Structure of abstract cortical task representations

Authors: ***S. SHRIDHAR**[1,2], H. XU[1], N. ZHOU[1,2], Z. LEVERONI[1,2], B. FENG[1], K. KHANNA[3], J. Y. YU[1,2];
[2]Inst. for Mind and Biol., [1]The Univ. of Chicago, Chicago, IL; [3]Univ. of Chicago Lab. High Sch., Chicago, IL

Abstract: Task-relevant representations are commonly found in the prefrontal cortex. Shared features in neural representations across tasks are thought to play a major role in supporting the generalization of shared rules across distinct tasks or contexts. However, the role of prior experience in shaping the transformation of neural representations across tasks is unclear. In this study, we investigate how prefrontal cortical task feature representations depend on prior experience. To examine neural representations in the prefrontal cortex, we recorded spiking activity using multi-tetrode recording drives. Rats were pretrained on one of two spatial tasks, thus creating two groups of animals with distinct prior experiences. Both groups were then trained to learn a new spatial navigation task in a new environment. For one group, the new task shared the rule of the familiar task (applicable). The other group of rats performed tasks that had different rules (non-applicable). We characterized several task feature representations based on the firing patterns across trajectories on the maze. We found that both groups formed distinct representations for the two distinct familiar tasks. For the novel task, we found a dominant task feature representation within a subpopulation of the cortical cells across applicable experiences. However, we found multiple task feature representations in subpopulations of cells across non-applicable experiences. Together, our results support the hypothesis that prior experience affects the properties of task representations in the prefrontal cortex.

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Poster

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Topic: I.08. Learning and Memory

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Title: Prefrontal cortical representations associated with learning multiple tasks

Authors: *N. ZHOU[1,2,3], H. XU[2], S. SHRIDHAR[1,2,3], Z. LEVERONI[1,2,3], B. FENG[2], K. KHANNA[4], J. Y. YU[1,2,3];
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Abstract: Learning involves dynamic reorganizations of neural circuits to accommodate new information. Individuals frequently encounter multiple learning experiences in rapid succession, where learning one task may facilitate, hinder, or have neutral effect on the acquisition of another. While both hippocampal and cortical regions are known to represent behavioral variables critical for goal-directed tasks, how these task representations are correlated with learning multiple experiences remains unknown. To address these questions, we designed an experiment where rats learned two mazes in an interleaved manner. We simultaneously recorded local field potential and spiking activity in the hippocampal dorsal CA1 and PFC regions over 5 days of learning and quantified the neural correlates of task structure in each maze. This allows us to characterize and compare the neural representations across tasks and determine how cortical neural patterns distinguish between tasks. We quantified the performance using a state-space model to estimate the probability of making a correct choice at each trial. All animals performed well in one task (~80%) but poorly in another (~50%) at the end of 5 days, suggesting that animals learned one task but had difficulty learning another. For the well performed task, cortical firing pattern exhibited consistent matching with a dominant task correlate. However, for the poorly performed task, cortical firing pattern exhibited matching with multiple task correlates. Our results suggest that a dominant, stable task representation is correlated with higher task performance, whereas the absence of a dominant, stable task representation is linked to lower task performance.

Disclosures: N. Zhou: None. **H. Xu:** None. **S. Shridhar:** None. **Z. Leveroni:** None. **B. Feng:** None. **K. Khanna:** None. **J.Y. Yu:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.11/PP3

Topic: I.08. Learning and Memory

Support: Brain Research Foundation BRFSG-2021-11
University of Chicago Neuroscience Undergraduate Honors Program

Title: Influence of prior experience on hippocampal sharp wave ripple dynamics during sleep

Authors: *A. E. KAYE[1], N. ZHOU[2,3], S. SHRIDHAR[2,3], Z. M. LEVERONI[2,3], J. Y. YU[2,3,1];
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Abstract: Hippocampal sharp wave ripples (SWRs) are critical for memory consolidation, particularly during NREM sleep. Sleep SWR rate is known to increase after a novel learning experience, while disrupting sleep SWRs impairs memory of novel experiences. However, it remains unclear what degree of novelty relative to prior experience is required to elicit changes in sleep SWR dynamics. We ask how related prior experience impacts the properties of NREM sleep SWRs across novel task learning. To address this question, we recorded CA1 LFP activity from rats learning two hippocampal-dependent maze-based memory tasks. We divided rats into two groups, which either had or did not have prior experience in any complex maze tasks. The first group of rats started the experiment without related experience, immediately spending five days training in two maze tasks across daily interleaved sessions. The second group initially gained related experience through five days of pre-training in a single maze task. Afterward, they spent another five days training in a new maze task alongside the familiar one through daily interleaved sessions. Animals had sleep sessions in between maze sessions throughout the experiment. To understand sleep SWR dynamics in these two groups with varied degrees of novelty, we quantified SWR properties—including rate, amplitude, and duration—during NREM sleep across training. We observed that rats without prior maze experience displayed increases in NREM SWR amplitude across sleep sessions on the first day of training, whereas those with prior experience did not. Our findings suggest that entirely novel task exposure triggers changes in sleep SWR dynamics, while task learning after related but non-identical prior experience may not.

Disclosures: A.E. Kaye: None. **N. Zhou:** None. **S. Shridhar:** None. **Z.M. Leveroni:** None. **J.Y. Yu:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: I.08. Learning and Memory

Support: NIH Grant R01MH112661
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Title: Complimentary hippocampal and prefrontal neural manifolds for memory schemas

Authors: *B. S. PORTER[1], C. SHI[2], S. P. JADHAV[1];
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Abstract: Animals live in dynamic environments where new information needs to be continuously extracted and consolidated from experiences. Memory schema, or relational networks of generalized knowledge, can facilitate the consolidation process by assimilating new information into existing memory networks based on common features. Furthermore, when faced with novel situations, animals can use contextual cues to retrieve related, abstracted information from schemas to infer appropriate actions and their outcomes. While a great deal of research has focused on memory schemas and inference, there is a gap in our understanding of the neural circuit-level physiological mechanisms that underlie schema formation, updating, and retrieval. Previous studies have demonstrated that the hippocampus and prefrontal cortex are critical regions for memory schema and inferential decision making. Using high-density electrophysiology, we investigated how neuronal activity in these two regions form, update, and retrieve memory schema (Long-Evans, male (N = 7) and female (N=1), 3-12 months old) using a novel spatial transitive inference task (A>B>C>D>E), where animals had to first learn the pair relationships (A>B, B>C, etc.) to form schemas, and were subsequently tested on inference for novel pairs (B>D). Transitive inference paradigms are especially useful as the schemata of the task must be constantly updated as new items are introduced during training, and then flexibly used when testing novel pairs. Using neural manifold analyses, we found that hippocampal representations form distinct representations of task elements (e.g. A, B, etc.), while adjacent elements (e.g. A-B) are most closely related. In contrast, prefrontal neural activity forms abstract, task-relevant action plans that generalize across items. Furthermore, we demonstrate that over learning, these abstract rule representations become more distinct as task performance improves. Overall, our results demonstrate that the hippocampus maintains a relational network of episodic information while the prefrontal cortex forms generalized policy decisions that can deployed in a context appropriate manner.

Disclosures: B.S. Porter: None. **C. Shi:** None. **S.P. Jadhav:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.13/PP5

Topic: I.08. Learning and Memory

Support: Simons Foundation (SFARI) Grant FN16-CC18700-PG02-GR403854

Title: Neural and Behavioral Mechanisms of Cooperation in Rat Models

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[3]Neurosci., Brandeis Univ., Somerville, MA; [4]Psychology, Brandeis Univ., Waltham, MA

Abstract: Cooperation is essential for survival in many species, often requiring detection of partner cues—such as location or actions—to coordinate toward a shared goal. Despite its importance, the behavioral and neural mechanisms underlying cooperation remain poorly understood—especially in naturalistic settings and in neurodevelopmental disorders like autism spectrum disorder (ASD), which are marked by social deficits. Here, we developed a novel spatial cooperation task to investigate the behavioral drivers of cooperation in wild-type (WT) and Fmr1-knockout (Fmr1-KO) rats—a model of Fragile X syndrome, the most common monogenic cause of autism and intellectual disability—and how hippocampal (dCA1) and prefrontal (mPFC) circuits—known to encode spatial and action information about self and others—support cooperative decisions. In this task, littermate rat pairs navigated opposing W-mazes separated by a transparent barrier and were trained to coordinate visits to matching reward wells to earn milk rewards. Animals were initially trained under a 100% joint reward contingency, in which every successful match was rewarded, and were later tested under a 50% probabilistic contingency to assess behavioral flexibility. Both genotypes learned the task, but WT pairs showed significantly higher coordination efficiency and synchrony, especially under probabilistic rewards. Control experiments showed both groups could associate visual cues with rewards and learn the 50% contingency in non-social versions of the task, suggesting that Fmr1-KO rats were specifically impaired in social coordination. Blocking visual access to partners disrupted coordination, and partner-directed attention modulated cooperative choices in WT—but not KO—rats, suggesting that rats rely on partner-derived cues for efficient coordination. Electrophysiological recordings from dCA1 and mPFC revealed that, during immobility periods at reward wells, ensemble activity was modulated during partner-directed orientation. Moreover, we observed reactivation of neural sequences representing spatial trajectories from both the subject's and partner's mazes, indicating cross-maze spatial representation. This led us to hypothesize that social attention dynamically biases dCA1-mPFC reactivation of behaviorally relevant spatial trajectories, thereby supporting cooperative decision making. Together, these findings establish a framework for linking social attention, spatial coding, and cooperative behavior at the neural circuit level, and for understanding how these processes may be disrupted in neurodevelopmental disorders such as Fragile X syndrome.

Disclosures: E. Rivera Melendez: None. **A. Shukla:** None. **J.D. Shin:** None. **S.P. Jadhav:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

Location: SDCC Halls B-H

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Program #/Poster #: PSTR413.14/PP6

Topic: I.08. Learning and Memory

Support: NIH Grant R01MH112661
NIH Grant R01DC020640

Title: Behavioral evidence of advanced planning in rats on a spatial alternation task

Authors: *C. W. REES[1], J. M. OLSON[2], S. P. JADHAV[1];
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Abstract: Deciding on an optimal plan before acting could reduce risk and energy expenditure while pursuing goals. In lab tasks, it is difficult for researchers to pinpoint the time when an animal makes its decisions. We examined whether male Long-Evans rats (n=9) display overt behaviors indicating trajectory plans prior to a choice point using a W-maze spatial alternation task. We restricted analyses to home arm working-memory decisions where rats needed to choose the next side arm based on the previously visited arm. We found that the direction rats turned when departing the home-arm reward well correlated to the side arm it visited next. Across trials (n=2484), 72.6% of clockwise home turns led to left arm choices and 70.3% of counterclockwise home turns led to right arm choices. In these cases, the home-arm turn oriented the rat's head direction towards its next destination before it initiated the trajectory, suggesting a possible sensorimotor component to the planning process. Furthermore, rats were more likely to correctly select rewarded options during matched-direction trials (86.7%) than during non-match trials (68.4%). These results suggest that rats plan trajectories before initiating them and that planning ahead increases their success.

Disclosures: C.W. Rees: None. **J.M. Olson:** None. **S.P. Jadhav:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR413.15/PP7

Topic: I.08. Learning and Memory

Support: NIH Grant R01MH120135

Title: Medial prefrontal cortical but not hippocampal representation extracts environmental structure by generalizing spatial features

Authors: *N. GIRIMAJI[1], K. FUKUMASU[1], J. YAMAMOTO[1,2,3];
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Abstract: The hippocampus and medial prefrontal cortex are critical for cognitive processes related to memory and navigation. Both these regions' representational content and their interactions during learning and mnemonic processes remain insufficiently characterized. We performed simultaneous high-density electrophysiological recordings using high density silicon probes (Neuropixels 2.0) in the hippocampus (HPC) and medial prefrontal cortex (mPFC) of freely moving mice (n=2) during square open-field exploration and memory-guided behavior.

Both HPC and PFC population activity significantly encoded animal position. Single unit Skaggs spatial information per spike and per second was higher in PFC than HPC ($p = 0.0015$) while HPC population decoding accuracy was significantly higher than that of PFC ($p < 2.2e-16$). The precise distribution of decoding error distances was visualized to elucidate the differences between HPC and PFC spatial representation. HPC exhibited a unimodal distribution centered around a peak error of 6.5 cm while PFC strikingly exhibited a bimodal distribution with peak errors of 2.5 cm and 75 cm, matching the side length of the square open field environment (72.4 cm). PFC demonstrated remote representation within the animal's length (10 cm) of this distance 19% of session time while the HPC demonstrated such representation 1.2% of the time ($p < 2.2e-16$). This effect, robust across sessions and animals, suggests PFC extracts environmental structure by generalizing spatial features.

Disclosures: N. Girimaji: None. **K. Fukumasu:** None. **J. Yamamoto:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Topic: I.08. Learning and Memory

Support: NSF DGE-1839285
NSF IOS-1150292
NIH R01-MH115697
NIH R01-DC017687

Title: Integrating hippocampal replay, prefrontal schemas, through the reinforcement learning of sequences

Authors: *K. W. COOPER[1], H. POURSIAMI[3], Z. YANG[1], M. PARSA[3], N. J. FORTIN[2]; [2]Dept. of Neurobio. and Behavior, [1]Univ. of California, Irvine, Irvine, CA; [3]George Mason Univ., Fairfax, VA

Abstract: Non-spatial sequence memory is crucial for navigating complex environments and making informed decisions. The hippocampus and prefrontal cortex are pivotal in these processes, with the hippocampus traditionally implicated in episodic memory and spatial navigation, and the prefrontal cortex in executive functions and flexible use of schemas. We recorded hippocampal activity as rats remembered an extended sequence of nonspatial events unfolding over several seconds, as in daily life episodes in humans. We found that the hippocampus's exhibits non-spatial sequence reactivation of past, present, and future odors during active sampling, as well as signs of awake replay of event sequences, suggesting its involvement in flexible processing and consolidation of sequences of events. Concurrently we find evidence of the prefrontal cortex's utilizing ordinal schemas, structured knowledge

representations that facilitate efficient sequence learning and prediction. We bridge these data by proposing an innovative computational model that connects hippocampal non-spatial replay with prefrontal action selection and schemas utilizing a reinforcement learning (RL) framework. This model, grounded in the anatomical circuitry of the hippocampus, was trained on a novel sequence task framework. We propose HiCoS (Hierarchical Contextual Sequences), a structured RL environment grounded in neuroscientific studies on event-sequence learning and context-driven decision-making. Taken together, this framework helps demonstrate how hippocampal replay mechanisms can drive the formation and flexible use of predictive schemas in the prefrontal cortex, thereby enabling flexible and adaptive behavioral strategies in complex, non-spatial environments.

Disclosures: K.W. Cooper: None. **H. Poursiami:** None. **Z. Yang:** None. **M. Parsa:** None. **N.J. Fortin:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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NSF IOS-1150292
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Title: Applying optogenetic approaches to test hippocampal-prefrontal causal relationships underlying the temporal organization of memories

Authors: *P. ASSAKURA MIYAZAKI[1], K. COOPER[2], G. A. ELIAS[3], N. J. FORTIN[2]; [1]Univ. of California Irvine, Irvine, CA; [2]Dept. of 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 of optogenetically manipulating HC → PFC and PFC → HC projections on rat behavior.

Disclosures: P. Assakura Miyazaki: None. **K. Cooper:** None. **G.A. Elias:** None. **N.J. Fortin:** None.

Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Program #/Poster #: PSTR413.19/PP10

Topic: I.08. Learning and Memory

Support: JST ERATO (JPMJER1801)
The Institute for AI and Beyond of the University of Tokyo
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AMED Brain/MINDS 2.0 (24wm0625502s0501)

Title: Neural activity in the hippocampus and frontal cortex in L-theanine-treated mice during the novel object recognition test

Authors: *K. WATANABE[1], K. TAKEDA[1], T. NAGAHIRO[1], S. IIJIMA[1], Y. IKEGAYA[2,3], N. MATSUMOTO[2,3];
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Abstract: L-theanine is a non-proteinogenic amino acid derivative originating from green tea leaves. As a natural analogue of glutamate, L-theanine exhibits high affinity for glutamate receptors. Upon oral ingestion, L-theanine is absorbed in the gut, transported into the brain, and functions as a glutamate receptor agonist by binding to glutamate receptors, thereby modulating excitatory neurotransmission. Given the critical role of glutamatergic modulation in the hippocampal function, it is reasonable to hypothesize that L-theanine administration influences memory and learning processes. Consistent with this view, previous studies have demonstrated that chronic administration of L-theanine enhances object recognition memory in rats through facilitated neurogenesis. While the cellular mechanisms underlying the L-theanine-induced enhancement in object recognition memory have been partially elucidated, the physiological bases remain largely unclear.To address this question, we chronically administered L-theanine (or tap water as control) to mice for three weeks and implanted electrodes into their hippocampus

and frontal cortex, regions crucial for object recognition memory. We allowed the mice to perform a novel object recognition task and recorded local field potentials (LFPs) in the two regions during the task. Task performance was evaluated using a discrimination index, defined as (N-F)/(N+F), where N and F represent time spent in the exploration of novel and familiar objects, respectively. LFPs in the hippocampus and frontal cortex were then analyzed by categorizing it into delta (1-4 Hz), theta (6-12 Hz), beta (18-25 Hz), low gamma (30-50 Hz), and high gamma (50-80 Hz) frequency bands. Absolute amplitudes for each frequency band were calculated using the Hilbert transform and normalized to the amplitude of a wide-frequency band during object exploration for comparison across training and test sessions.We found that mice administered with L-theanine exhibited a higher discrimination ratio compared to control mice. Furthermore, theta oscillations in the frontal cortex and beta and low gamma oscillations in the hippocampus were enhanced during exploration of familiar objects in L-theanine-treated mice. These findings highlight novel physiological underpinnings of the enhancement of object recognition memory mediated by exogenous substances.

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Poster

PSTR413: Hippocampal-Cortical Networks: Neurophysiological and Behavioral Studies in Rodents

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Program #/Poster #: PSTR413.20/PP11

Topic: I.08. Learning and Memory

Support: IBS-R002-A1

Title: Value-dependent reactivation of navigational experiences in the prefrontal cortex and hippocampus

Authors: *G. YOUN[1,2], M. W. JUNG[1,2];
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Abstract: Memory consolidation is thought to involve the transfer of newly encoded information from the hippocampus to distributed neocortical networks. However, the principles governing which experiences are prioritized for consolidation remain unclear. We hypothesized that the brain preferentially consolidates high-value experiences over low-value ones. To test this, we examined whether navigational experiences associated with different reward probabilities are uniformly or selectively reactivated in the overtraining stage. Using simultaneous recordings from the medial prefrontal cortex (mPFC) and intermediate hippocampus (iHPC) in mice navigating three virtual environments associated with different reward probabilities, we examined neural activity during hippocampal sharp-wave ripples—events critical for memory

consolidation. We found that high-value contexts were preferentially reactivated in the mPFC, whereas low-value contexts were more strongly reactivated in the iHPC. Notably, this value-dependency was observed not only during task engagement, but also during pre- and post-task offline periods. These region- and context-dependent reactivations suggest that hippocampal ripples recruit distributed, value-sensitive circuits across brain regions. Hippocampal ripples may thus provide a temporal window for selective memory consolidation based on behavioral relevance.

Disclosures: G. Youn: None. **M.W. Jung:** None.

Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

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Topic: I.08. Learning and Memory

Support: C1 (C14/22/134)
Mission Lucidity

Title: Effects of epicranial electrical stimulation on spiking and local field potential activity in the hippocampus of non-human primates

Authors: *N. PEELEMAN, P. JANSSEN;
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Abstract: Neuromodulation holds promise to enhance neurological and cognitive functions and gained interest as treatment for various neurological disorders. We previously demonstrated the potential of epicranial current stimulation (ECS, i.e. on the skull) to improve spatial memory and to activate multiple brain regions, including the hippocampus in non-human primates (Peeleman, N (2023, Feb 20-22) *Selective hippocampal activation and memory enhancement caused by epicranial electrical stimulation*, International Brain Stimulation Conference, Lisbon, Portugal). However, the neural effects of ECS remain unclear. To investigate this, we recorded single-unit activity (SUA) and local field potentials (LFPs) from the hippocampus of two rhesus macaques during a passive fixation task and a spatial memory task while applying electrical stimulation on chronically implanted epicranial electrodes. In the passive fixation task, hippocampal activity decreased during 40 Hz ECS in two monkeys and did not fully recover after stimulation offset (one-way ANOVA, $p < .001$). On the other hand, 10 Hz ECS only weakly reduced spike rate during stimulation in M1 (one-way ANOVA, $p = .012$) and had no significant effect in M2 (one-way ANOVA, $p = .16$). A two-way ANOVA to test whether the effect of 40 Hz ECS differed from 10 Hz ECS revealed significant main effects of frequency (M1: $p < .001$; M2: $p = .0003$) and time period (baseline, stimulation or poststimulation, M1: $p < .001$; M2: $p = 0$) and a significant interaction (M1: $p < .001$; M2: $p = .0008$). In the spatial memory task, the monkey had to remember the location of two circles on a touch screen. We applied ECS during

presentation of half of the patterns while the monkey performed the task. Similar to the passive fixation task, 40 Hz ECS significantly decreased the delay period activity of neurons (N=22) for stimulated patterns (three-way ANOVA, significant effects of stimulation and time period, post hoc Tukey test, $p = .0001$). This reduction in delay period activity recovered in the next block, where no stimulation was delivered. At the LFP level, both nonstimulated and stimulated patterns induced a strong decrease in alpha (8-12 Hz) and beta (12-25 Hz) power during target presentation. However, in the delay period of the stimulation block, stimulated patterns showed stronger theta (4-8 Hz), alpha and low gamma power (25-50 Hz) compared to nonstimulated patterns (permutation test, $p < .05$). Thus, 40 Hz ECS primarily induces a reduction in neural activity in the hippocampus in macaque monkeys.

Disclosures: N. Peeleman: None. **P. Janssen:** None.

Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

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NINDS-UF1NS126485 (E.A.B, A.L.F.)

Title: Emergence of task-specific value representations in the primate hippocampus

Authors: *S. M. LANDI[1,3], V. SHIRHATTI[4], D. J. FREEDMAN[5], A. L. FAIRHALL[2], E. A. BUFFALO[2,3];
[2]Neurobio. and Biophysics, [1]Univ. of Washington, Seattle, WA; [3]Washington Natl. Primate Res. Ctr., Seattle, WA; [4]Dept. of Neurobio., [5]Neurobio. and Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: The hippocampus is known to encode task-related variables, from classic place cells to tone-tracking and value place cells. However, it is unclear how such representations evolve during learning. Studying this in primates has been particularly challenging due to the lack of suitable paradigms and limited access to high-density neural recordings. Here, we recorded the activity of large hippocampal populations in macaques learning a set of naturalistic foraging tasks in a virtual reality environment. Using a joystick, monkeys navigate in an open-field virtual arena and sequentially harvest rewards by colliding with visual targets. Each recording session includes two interleaved tasks: one in which reward value is determined by target position

(spatial task), and another by target color (color task). Critically, on each trial, monkeys are given a limited time to harvest targets, such that they can only harvest about half of the targets in the arena before they disappear. Therefore, learning the underlying value association leads to more efficient foraging and greater reward. Each recording session, monkeys learn novel color- and space-value associations. Unlike other behavioral tasks that can take many months for monkeys to learn, our paradigm enables efficient and robust learning within a single recording session, making it possible to track learning-related changes in hippocampal activity in real time. We recorded hippocampal activity using 32- and 64- channel Plexon S-probes and 1.0 NHP Neuropixels along the anterior-posterior axis of the hippocampus while monkeys performed the foraging tasks. We examined representations of sensory inputs, their associated values, and the abstract task structure both at the level of single units and the simultaneously recorded neural ensemble. We used principal component analysis to identify the leading covariance patterns in the population. Although individual neurons showed complex mixed selectivity, a task-dependent low-dimensional structure in the neural population emerged during learning: initially overlapping representations gradually differentiated into distinct subspaces, each associated with a specific task and value map. The hippocampus thus emerges as a key node in linking crucial aspects of ongoing experiences with their outcomes, weighting reward value maps in a task-dependent manner.

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Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

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Title: Semantic parsing of experience by discrete neural states in the monkey hippocampus

Authors: *J. W. RUECKEMANN[1], Y. I. BROWNING[2], A. MALLORY[1], B. KIM[3], A. L. FAIRHALL[1], E. A. BUFFALO[1];
[1]Univ. of Washington, Seattle, WA; [2]Allen Inst. for Neural Dynamics, Allen Inst., Seattle, WA; [3]Univ. of Michigan Neurosci. Grad. Program, Ann Arbor, MI

Abstract: The hippocampus exhibits structured responses across diverse behavioral tasks, fueling debate about its generalized function. While spatial selectivity has dominated accounts of hippocampal activity, growing evidence suggests that task structure—particularly the sequence

of salient behavioral events—may fundamentally organize hippocampal coding.
We analyzed neuronal activity recorded from the full extent of the hippocampus using chronic microdrives in three rhesus macaques as they performed a virtual delayed spatial alternation task. Approximately 30% of neurons exhibited reliable task-related activity. These responses were not uniformly distributed in allocentric space, as would be expected with canonical place cells. Instead, the temporal boundaries of spiking tended to align to salient behavioral events (movement start, choice, reward, and intertrial interval), suggesting event-based segmentation. Analysis of aggregated pseudopopulation activity revealed discrete clusters in neural state space corresponding to behaviorally defined epochs. Salient events coincided with sharp transitions between these clusters, marked by an acute drop in cross-trial variability that suppressed noise while preserving event-linked spiking. Within each epoch, neural activity was concentrated in a distinct region of state space. While fine-timescale activity within each epoch varied from trial to trial, transitions across epochs were highly consistent—indicating that population dynamics were locally stochastic but globally structured. Bayesian decoding precision and accuracy peaked at event boundaries indicating that they were the most informative moments in the trial. While decoding individual time bins within behavioral epochs was unreliable, the identity of the epoch itself was robustly decoded—suggesting a categorical, rather than continuous, organization of hippocampal representations.
This segmented coding scheme was conserved across visually distinct environments. Despite changes in sensory input, many neurons maintained stable, event-aligned responses, and a decoder trained in one environment generalized robustly to others. These data demonstrate that the hippocampus encodes an abstract, task schema—a simplified blueprint capturing the ordinal relationships among salient behavioral states.
Together, these findings indicate that hippocampal population activity in primates forms an event-anchored scaffold for tracking task experience. Rather than encoding continuous space or time, these data suggest that the hippocampus segments behavior into discrete, relational states that reflect learned task structure.

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Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.04/PP15

Topic: I.08. Learning and Memory

Support: NSF GRFP 2023356971
NIH 5U19NS107609
Simons Collaboration for the Global Brain

Title: Distributed belief representations support flexible inference and decision making under uncertainty

Authors: *P. Q. ZHANG[1], M. J. JUTRAS[2], E. A. BUFFALO[2], E. Y. WALKER[3], A. L. FAIRHALL[4];
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Abstract: To navigate a complex and uncertain world, one often needs to maintain and update beliefs, estimates of the hidden aspects of the world that are relevant to the task at hand.The hippocampus, inferior temporal cortex, and basal ganglia have been classically implicated in memory, object recognition, and decision making respectively, yet how activity in these regions is integrated to contribute to belief representation remains unknown. To address this, monkeys were trained to perform a variant of the Wisconsin Card Sorting Task (WCST), which required them to repeatedly choose cards from a screen in which, to be successful, they needed to infer a hidden rule (a color, shape or pattern of the card) through trial and error. After choice is indicated by a 800ms fixation, cards disappear from the screen while feedback is delivered (food or a time-out). A belief state behavioral model supported the hypothesis that monkeys perform the task by maintaining belief distributions of the hidden rule, summarized by their overall confidence in their belief, and a preference towards one rule or the other. On a trial-by-trial basis, beliefs are then updated by integrating the current trial's sensory evidence of card choice and feedback with prior beliefs. Using single unit recordings from all three regions, we find that belief confidence and belief preference are linearly decodable from population activity throughout the trial, with decoder weights distributed across all three regions. The neural representation, as inferred from decoding weights, was more dynamic during the decision making phase of the trial, and more stable from the end of the current trial's feedback period to the next trial's initiation, suggesting distinct population codes for belief-guided action and belief maintenance. Furthermore, we observe robust representations of card features, choice and feedback, as well as choice-feedback interactions during the feedback period of the trial, implicating all three regions in belief updating. Together, these findings suggest that the hippocampus, inferior temporal cortex, and basal ganglia together dynamically represent and update task-relevant beliefs to support flexible behavior under uncertainty.

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Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.05/PP16

Topic: I.08. Learning and Memory

Title: Two-month neural tracking reveals divergent stable and emergent features of neural geometry between rat HP and mPFC throughout learning a task in VR

Authors: *S. J. STEFFENS[1], Y. LIU[2,4], H. YAO[3,5], P. GOMBKOTO[7], A. L. VYSSOTSKI[9], H. JAVAHERI[6], E. ÖZIL[8], T. YASAR[10], W. VON DER BEHRENS[11], M. F. YANIK[12]; [1]ITET, ETHZ, Zürich, Switzerland; [2]Zurich, ; [3]Geneva, ; [4]HiFo, Univ. Zurich, Zurich, Switzerland; [5]Neuro-X Inst., EPFL, Geneva, Switzerland; [6]EPFL, Lausanne, Switzerland; [7]ETH Zürich, Zürich, ; [8]ETH Zürich, Zurich, ; [9]Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, ; [10]ETH Zurich, Zurich, ; [11]Univ. of Zurich and ETH Zurich, Zurich, ; [12]ETH, Zurich.

Abstract: Learning involves coordinated activity across distributed brain regions, yet how this coordination unfolds over time remains unclear. While both the hippocampus (HP) and medial prefrontal cortex (mPFC) are known to contribute to memory and decision-making, it is unclear over what trajectories their neural population dynamics evolve and interact throughout learning in a moderately complex task. Using our ultra-flexible tentacle electrodes (UFTEs), we tracked the same neurons in HP and mPFC over two months as rats learned a cue-based two-alternative choice task in a body-fixed virtual reality setup. By bonding our electrodes to a CMOS array, we recorded 1024 channels simultaneously, first tethered (task in VR), followed by a freely moving session (sleep) using a newly developed logger system. With a total weight of 12g and a battery capacity of 350 mAh, our logger could record neural activity of 1024 channels at 20 kHz for 2.6h with ultra-low noise. Over the course of 2 months, rats learned to associate a visual cue in the early region of a linear track with stopping in two distinct regions later on the track. We identify distinct behavioral strategies expressed by the animal over intervals ranging from minutes to days, tracing a trajectory of learning in behavioral space. Importantly, we relate these evolving strategies to both stable and plastic components of the low-dimensional geometry of population activity in HP and mPFC. By analyzing the canonical angles between principal component subspaces across sessions, we distinguish conserved directions of neural variance from those that reorganize over time, revealing how consistent and flexible dimensions of neural population dynamics underlie behavioral change. Together, these findings demonstrate how the stability of high-density, distributed UFTE recordings enables the investigation of long-timescale dynamics of neural circuits during learning.

Disclosures: S.J. Steffens: None.

Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR414.06/PP17

Topic: I.08. Learning and Memory

Support: Howard Hughes Medical Institute

Title: Closed-loop spatial memory discrimination task identifies behaviorally accessible population-level non-local representations in the rat hippocampus

Authors: *D. SHIN[1], S. CHIANG[1], E. BROYLES[1], M. E. COULTER[1], K. LEE[1], A. M. YORITA[2], J. HERNANDEZ[2], R.-U. HAQUE[2], L. M. FRANK[1];
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Abstract: A hallmark of human memory recall and imagination is the ability to access self-generated neural content to guide behavior. Lesion and neuroimaging studies have shown that the hippocampus is critical for both memory recall1,2 and imagination3-5, yet the neural mechanisms at the level of populations of neurons underlying these processes remain unclear. In rodents, hippocampal place cell reactivation has been proposed as potential mechanism supporting recall6-8 and imagination9,10,but it remains unknown whether such reactivation content is accessible to guide subjects' behavior.To address this, we developed a closed-loop spatial memory discrimination task, a two-choice task in which correct choices are based on subjects' internally generated neural activity, to test whether rats can behaviorally access the content of hippocampal place cell reactivation. In the task, rats navigated a Y-maze while we chronically recorded spiking activity from hundreds of hippocampal neurons11. During the test phase, rats remained in the maze center. A real-time decoder trained to decode the rat's position using population activity was used to detect remote representations of two target locations, each one meter away from the rat at the time of detection11-13. When a representation of either target location was detected, the animal was cued to initiate navigation, but was not cued which arm to navigate to. The animal was rewarded only if it navigated to the represented arm.All four rats learned the task, achieving performance significantly above chance (mean accuracy up to 78%; chance = 50%). Head direction at the time of detection did not correlate with performance, suggesting that choice was guided by internal neural signals rather than postural or other sensorimotor cues. Additionally, trials with shorter response times showed higher accuracy, echoing results in the perceptual decision-making literature14. Critically, we identified consistent coactivation of place cells along the path to the represented target location. Moreover, we observed coactivation of held-out neurons tuned to target but not used for real-time decoding. These findings indicate that, to perform the task, rats did not simply learn arbitrary neural activity patterns but accessed endogenous place cell representations of each of the target locations.Together, these findings demonstrate that remote hippocampal representations are behaviorally accessible in rats, causally supporting hippocampal reactivation as a physiological substrate for memory recall and imagination.

Disclosures: D. Shin: None.

Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR414.07/PP18

Topic: B.07. Network Interactions

Support: NIH Grant R01

Title: Simultaneous excitatory inputs from the lateral and medial entorhinal cortices to the dentate gyrus during dentate spikes

Authors: *G. TARCSAY[1,2], R. SAXENA[4,3], R. LONG[1], N. MASALA[2], B. L. MCNAUGHTON[5,3], L. A. EWELL[2];
[2]Anat. & Neurobio., [3]Neurobio. & Behavior, [1]UC Irvine, Irvine, CA; [4]Neurobio. and behavior, Kavli Inst. for Systems Neuroscience, NTNU, Norway, Trondheim, Norway; [5]Dept. of Neurosci., The Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Dentate spikes (DSs) are large, positive deflections in the dentate gyrus local field potential, initiated by the entorhinal cortex (EC). Typically, DSs are classified based on their current-source density profile. One type exhibits a current-sink in the outer molecular layer, suggesting a lateral EC input (DS1), while the other type is accompanied by a current-sink in the middle molecular layer, indicating a medial EC input (DS2). Although the classification method has been widely utilized, it relies on early findings of two distinct types of DSs that were recorded with silicone probes with low spatial resolution (100 µm) and thus might disregard events with simultaneous lateral and medial EC excitation. To test this hypothesis, we recorded dentate spikes in awake, resting mice (N=8, 0.37±0.08 Hz mean± sd event rate) using silicone probes with fine spatial resolution (20-25 µm), and developed a novel classification method that allows to distinguish DSs with two current-sinks. Strikingly - beside observing DS1s and DS2s (0.10±0.05 Hz and 0.17±0.08 Hz) - we found a substantial portion of DSs that were characterized by current-sinks in both molecular layers and we refer to them as DS3s (0.10±0.06 Hz). We further found that the magnitude of the current-sinks during DS3s are highly variable, forming a continuum between DS1s and DS2s. Next, we investigated dentate spikes in mice during deep sleep and found that while DS2 rate increased compared to awake mice (N=5, 0.49±0.12 Hz, p=0.004, Wilcoxon rank-sum test with post-hoc Bonferroni correction), DS3 rate was reduced (0.03±0.01 Hz, p=0.009), as well as DS1 rate (0.03±0.01 Hz, p= 0.005), supporting previous findings of suppressed LEC activity during deep sleep. These findings suggest that DSs may be elicited as a continuous function of lateral and medial EC excitation in a brain state dependent manner. Receiving simultaneous signals from the lateral EC (non-spatial, context-related information) and from the medial EC (spatial information) may support binding distinct information on the time scale of several milliseconds. We currently perform probe recordings during a hippocampal-dependent context to reward location association task to further understand cortico-hippocampal interactions during DSs.

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Poster

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Program #/Poster #: PSTR414.08/PP19

Topic: I.08. Learning and Memory

Support: NRF2021R1A2C3005560
NRF2019R1A6A3A01096971

Title: Memory trace of a virtual butterfly encounter

Authors: *D. JUNG, S. ROYER;
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Abstract: Episodic memory is necessary for animals to adapt to the dynamically changing world. A substantial body of research has described how the hippocampus updates its representation of the external world in response to novel cues. However, the mechanism that enable the parallel processing and integration of familiar spatial information and novel transient information is still unclear. In this study, we developed a novel paradigm in which head-fixed mice run on a cue-enriched treadmill and occasionally encounter a butterfly dummy controlled by 3-motor axes, and we recorded neuronal responses in hippocampal CA1 and CA3 regions using silicon probes. We found that the f irst encounter with the butterfly rapidly remodeled the activity patterns of place cells. While the prior encoding of treadmill belt locations could still be observed in the population code of gamma-scale time windows, new population activity patterns emerged and recurred during the butterfly encounters, multiplexing with spatial information. Furthermore, a neural network model of competitive learning effectively replicated the range of place cell responses and revealed a parallel development of engrams for both spatial environments and butterfly encounters. These results demonstrate the existence of hippocampal memory traces for single encounter events and provide insights into the neural network mechanisms that support the 'one-shot' encoding of experiences

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Poster

PSTR414: Learning and Memory: Hippocampal Neurophysiology

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Program #/Poster #: PSTR414.09/PP20

Topic: I.08. Learning and Memory

Support: KAKENHI 25K18591

Title: A hippocampal-septal pathway mediates bidirectional control of memory

Authors: *H. HE, T. J. MCHUGH;
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Abstract: The reciprocal connectivity between the hippocampus and medial septum (MS) plays a critical role in integrating sensory and motor information during learning, primarily through the generation of theta oscillations. While the ascending cholinergic, glutamatergic, and GABAergic projections from the MS to the hippocampus have been well characterized, the function of

descending hippocampal efferents remains poorly understood. Here, we combine transgenic and viral approaches, in vivo electrophysiology, and optogenetic manipulations to identify and functionally dissect a previously uncharacterized descending projection from hippocampal CA2 pyramidal neurons to the MS. Anatomical tracing reveals that approximately 6-10% of CA2 pyramidal cells form monosynaptic excitatory connections with MS GABAergic neurons. Optogenetic activation of CA2 axons in the MS recruits this GABAergic population, which in turn gates local cholinergic and glutamatergic neurons. Functionally, modulation of CA2 terminals in the MS bidirectionally regulates septal cholinergic tone and hippocampal network states, dynamically shifting between theta and ripple oscillations. These manipulations also induce bidirectional changes at both single-cell and population levels, underscoring a role in memory-related temporal coding. Behaviorally, activation of the CA2-MS projection impairs memory performance, while inhibition enhances memory discrimination. Together, these findings demonstrate that CA2 exerts regulatory control over septo-hippocampal dynamics, shaping information processing during distinct phases of memory encoding and consolidation. This study reveals a novel function of CA2 in orchestrating hippocampal "online" and "offline" memory states, providing new insight into the mechanisms by which long-range hippocampal outputs regulate mnemonic processing.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR415.01/QQ1

Topic: I.09. Spatial Navigation

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Title: Impairment of medial entorhinal parvalbumin interneurons in a knock-in model of Alzheimer's disease

Authors: *J. XIE[1], H. JUN[2], T. C. SAIDO[3], K. M. IGARASHI[1];
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Abstract: Alzheimer's disease (AD) patients suffer from spatial memory impairment and wandering behavior, but its underlying neural circuit mechanism remains unclear. Using the

amyloid precursor protein knock-in (APP-KI) mice, we previously found that grid cells activity in the medial entorhinal cortex (MEC) was impaired earlier than the impairments of CA1 place cell remapping and spatial discrimination memory (Jun et al., Neuron 2020). This suggested that the earlier impairment of MEC grid cells underlies spatial memory impairment, but the mechanism of how the MEC grid cell becomes impaired remains unknown. We recorded parvalbumin (PV)-expressing interneurons in the MEC of APP-KI mice using opttag recording and observed that PV interneurons showed decreased firing. Chemogenetic reactivation of PV cells in APP-KI mice improved grid cell coding of MEC neurons and rescued their spatial memory performance in a path integration task. These results suggest that the dysfunction of MEC PV cells underlies grid cell impairment and spatial memory deficit of APP-KI mice.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR415.02/QQ2

Topic: I.09. Spatial Navigation

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Title: Topographical organization of functional cell types in the medial entorhinal cortex

Authors: *M. POFAHL, N. DE JONG, H. A. OBENHAUS, H. ENEQVIST, M. P. WITTER, W. ZONG, M.-B. MOSER, E. I. MOSER;
Kavli Inst. for Systems Neurosci., Trondheim, Norway

Abstract: The medial entorhinal cortex (MEC) encodes spatial information through a set of canonical cell types, including grid cells, object-vector (OV) cells, head-direction (HD) cells, and border cells. While these functional cell types are co-located within the superficial layers of MEC and the adjacent parasubiculum (PaS), the exact anatomical distribution of these cell types within the MEC remains to be determined. Previous studies showed that grid cells form discrete modules of increasing scale along the dorsal-ventral axis of MEC. Additionally, imaging studies found a non-uniform distribution of grid cells across MEC and suggested a clustering of grid cells towards the dorsomedial MEC, whereas OV, HD, and border cells appeared more uniformly distributed. However, technical limitations have restricted the simultaneous mapping and quantification of these cell types across extended anatomical regions in individual animals.

To overcome these limitations, we performed two-photon calcium imaging via miniscopes (MINI2P) and combined multiple field of views (FOVs) obtained in freely moving mice across a 1.3mm wide square prism implant covering MEC and PaS. The stitching of FOVs combined with multi-plane imaging allowed us to record and map neural activity across layer II in dorsomedial MEC and PaS. The imaging area includes the three most dorsal modules with all of module M2 and parts of dorsal M1 and ventral M3. Each grid cell module forms a distinct anatomical cluster (´hot spot´) in the dorsomedial MEC, near the PaS border. This ~300 um wide band of grid cell modules is anticorrelated to the anatomical locations of other functional cell types. The estimated fraction of grid cells in the M2 hot spot of layer II is in the order of 30%. Scattered grid cells were observed outside the hot spot often exhibiting irregular grid patterns. We find more and sharper tuned HD cells in PaS but also find a stable fraction of more weakly head direction modulated cells across MEC, often in conjunction with other functional properties. We find border cells and OV cells in both PaS and lateral MEC. However, OV cells in MEC possess longer vectors between animal and object and a stronger anatomical clustering than the OV cells of PaS. Our data will allow us to define a general functional map of the superficial layers of MEC and PaS and ultimately quantify the total number of layer II grid cells for one module. Our findings reveal a previously unrecognized tight anatomical clustering of grid cells and suggest a structured spatial relationship between grid and non-grid cells, with implications for the organization and integration of spatial signals within the entorhinal-hippocampal circuit.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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National Infrastructure grant from Research Council of Norway (NORBRAIN, grant 295721)

Title: Do grid cells track elevation changes?

Authors: *V. KARGÅRD OLSEN[1], M. SCHELLENBERGER[1], A. LAUTRUP[1], D. HAYDEN[1], B. DUNN[2], M.-B. MOSER[1], E. I. MOSER[1];

[1]Kavli Inst. for Systems Neurosci., Trondheim, Norway; [2]Dept. of Mathematics, Trondheim, Norway

Abstract: The hexagonal pattern of grid cells, and the resulting toroidal manifold, are well understood in flat 2D environments (Gardner et al., 2022). This system appears adapted to optimally represent positions in a plane. However, it has not been determined how grid cells and their toroidal manifolds support surface or volumetric navigation in 3D. Previous work in rodents has investigated this using ramps, walls, 3D mazes, or other environments with distinct boundaries, providing the grid cells with ample opportunities to realign the grid phase and orientation. Previous work in flying bats has pointed to an aperiodic 3D representation, though these studies also have salient boundaries or landmarks that might induce realignment, such as landing balls or the presence of an experimenter. Finally, most previous work has been on the single cell level, potentially obscuring how grid cells coherently adapt to the 3D environment.
We address these issues by studying how grid cell population activity traverses the torus as mice navigate a big spherical cap. To do this, we use high-density Neuropixels silicon probes to record from large numbers of cells in the medial entorhinal cortex, during free exploration of the dome. The surface of the dome is continuous and thus provides no obvious opportunity for the grid cells to realign. If the grid cells remain tightly linked to distance travelled over the entire dome, the localized firing fields should be washed out. We might expect the grid cells to solve this by (1) arbitrarily breaking up the environment into different locally flat alignments or (2) morphing and adapting to the sphere, potentially sacrificing the hexagonal pattern.
In preliminary data from a single mouse with about a thousand neurons, including more than a hundred grid cells from three grid modules, we don't see evidence of either strategy. For grid cells from the two larger modules, the firing fields on the dome are organized hexagonally when seen from above, just like in flat environments (mirroring grid cell rate maps in a helical maze in Hayman et al. 2011). This suggests that the torus is aligned to the xy-plane, which is confirmed by the toroidal phase changing linearly in the x- and y-direction, seemingly ignoring the z-direction and implying that grid cells don't simply track distance travelled. For the smallest grid cell module, in contrast, the firing fields and the resulting toroidal decoding are less clear. This could be due to too few cells from the smallest module, or alternatively it might suggest a scale-dependent effect of environmental curvature. Data is being collected from additional mice, and from intermixed speed cells, to determine the generality of the findings.

Disclosures: V. Kargård Olsen: None. **M. Schellenberger:** None. **A. Lautrup:** None. **D. Hayden:** None. **B. Dunn:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Topic: I.09. Spatial Navigation

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National Infrastructure grant from Research Council of Norway (NORBRAIN, grant 295721)

Title: Dynamics of remapping across the hippocampal-subicular-entorhinal circuit

Authors: *J. CARPENTER[1], M. GUARDAMAGNA[1], C. LYKKEN[1], R. J. GARDNER[1], B. DUNN[2], E. I. MOSER[1], M.-B. MOSER[1];
[1]Kavli Inst. for Systems Neurosci., Trondheim, Norway; [2]Dept. of Mathematics, Norwegian Univ. of Sci. and Technol., Trondheim, Norway

Abstract: The hippocampal-subicular-entorhinal circuit supports the formation and retrieval of spatial maps. When an animal enters a new environment, hippocampal place cells typically globally remap, while grid cells in medial entorhinal cortex (MEC) and head direction and border cells in the subicular complex shift or rotate their tuning but maintain internal structure. Although remapping has been extensively studied, most work has focused on single regions, small populations, and pairs of environments, rarely capturing the dynamics of transitions between contexts. In addition, the subiculum remains underexplored, despite its reciprocal connections with both CA1 and MEC and its potential role in transforming or relaying contextual signals. In this study, we performed large-scale recordings across the circuit using four Neuropixels 2.0 probes in rats navigating five distinct environments. A mobile cart system enabled continuous neural recording during transitions, allowing us to examine how population activity in CA1, MEC, and the subicular structures reconfigures across rooms and whether remapping is triggered by changes in sensory information, active locomotion, or both. As expected, CA1 place cells and the combined activity of multiple MEC grid modules formed distinct representations across rooms. In contrast, single grid modules, dorsal pre-subicular head direction cells, and border cells retained their relative tuning across environments. In all these regions, population activity was reinstated during repeated visits to the same room, and accurate room decoding in CA1 and MEC emerged only once the animal began to explore. Interestingly, subicular activity formed a structured pattern in low-dimensional space that reflected the sequence of room visits, with reliable decoding already present the moment the animal entered a new room- minutes before active exploration through the open field began. Additionally, decoder errors in subiculum often pointed to previously visited rooms, while future rooms were rarely anticipated. These findings suggest that the subiculum may contribute to the organization of sequential experiences by linking nearby events through partially overlapping population activity patterns. Ultimately, we aim to uncover how spatial context (room) is encoded, updated, and coordinated across the circuit by identifying where remapping is first initiated, which subpopulations are engaged, and how transitions between rooms unfold at fine timescales.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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EMBO Postdoctoral Grant (ALTF 738-2022)

Title: The ontogeny of toroidal manifolds and the grid cell system

Authors: *M. GUARDAMAGNA[1], E. HERMANSEN[2], J. CARPENTER[3], C. LYKKEN[3], B. DUNN[2], E. I. MOSER[3], M.-B. MOSER[3];
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Abstract: The medial entorhinal cortex (MEC) serves as a central hub for the coding of spatial experiences. Grid cells and head direction (HD) cells are essential elements of the navigation system. At the population level, the activity of these cells traverses low-dimensional manifolds - torus and ring manifolds respectively - consistent with the predictions of continuous attractor network theories for these cells. Although spatial coding dynamics have been extensively studied in adult rodents, how large neural populations organize during development is not well understood. In the present study we investigated the emergence and maturation of the grid and head direction networks in rat pups. Using chronic Neuropixels 2.0 recordings, we tracked large populations of entorhinal neurons (400-1200 simultaneously recorded units) in animals as early as postnatal day 8. We discovered entorhinal ensemble activity with toroidal topology as early as postnatal day P10, before active exploration outside the nest and even before eye and ear-canal opening. Ensembles with toroidal topology could not be detected on postnatal days 8 or 9. The abrupt emergence of toroidal manifolds aligned with a desynchronization of entorhinal population dynamics, which in turn coincided with an increase in inhibitory drive within the network, enabling the entorhinal cortex to disengage from external sensory inputs.
In adult animals, grid cells are organized into semi-independent modules, each one with a distinct grid spacing. In rat pups, we identified the presence of multiple toroidal modules from the beginning, as early P10, topographically organized along the dorsoventral axis of the MEC. Although modules seemed to be preconfigured, their dynamics retained a certain degree of correlation until P12 - reflecting a gradual emergence of independence between the modules. From P15 onward we observed the gradual emergence of grid fields, with reference to physical location, in individual cells of toroidal ensembles; however, regularity in the grid pattern

emerged only towards the end of the 3rd postnatal week - reflecting a slow, experience-dependent mapping of the torus onto the external environment. Finally, we detected ring manifolds in para-subiculum as early as P9, before tuning of individual cells to head direction. Taken together, these observations suggest that the neural network architectures for the coding of space have strongly experience-independent components, with self-organizing principles guiding the formation of low-dimensional manifolds that are later mapped onto physical space.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Topic: I.09. Spatial Navigation

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Title: High-yield two-photon calcium imaging in freely moving mice

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Abstract: Decoding how large-scale neuronal networks encode behavior, memory, or spatial navigation requires chronic, cellular-resolution recordings across wide brain regions in naturalistic settings. Silicon probes offer millisecond temporal precision in large populations but lack genetic specificity, subcellular resolution, and selective manipulation of defined neurons. One-photon miniscopes eliminate head fixation yet compromise optical sectioning, restricting them to sparse labelling samples. Current two-photon (2P) head-mounted devices—including MINI2P (Zong, et.al. 2022) and the fiber-bundle 2P-FENDO (Accanto, et.al. 2023)—either image a small field of view (FOV) or trade resolution for throughput, limiting recordings near the hundred-cell scale. Therefore, achieving simultaneous recording of thousands of neurons with high spatial resolution, recording density, and imaging speed remains beyond the capability of existing miniscopes.Here, we present a next-generation 2P miniscope, engineered to reach the ten-thousand-neuron recording yield regime in freely moving mice. Key innovations include: (*i*) A high-NA (0.45), large-aperture, mini-objective supporting up to 1 mm-diameter FOV—4 times

larger the FOV of MINI2P—while maintaining high spatial resolution; (*ii*) a large-angle scanning (±8°) MEMS mirror—twice the range of MINI2P scanner; (*iii*) a compact, high-bandwidth on-board silicon photomultiplier (SiPM) circuit with wide-angle collection optics; and (*iv*) a multi-beam, spatio-temporal multiplexing device for simultaneous multi-plane imaging. Using this new 2P miniscope with a single excitation fiber, we recorded high signal-to-noise-ratio (SNR) calcium signals from ~1,000 neurons in a single 1 mm-diameter FOV in layer II visual cortex of freely behaving mice (GCaMP6s transgenic line expressing in all excitatory neurons). This cell yield significantly exceeds those of current state-of-the-art 2P miniscopes under similar imaging speed, depth and labeling conditions. Integration of the spatiotemporal multiplexing system with multiple excitation fibers further allows simultaneous imaging across depth-offset planes, increasing cell yield without compromising speed or SNR. By scaling 2P imaging to mesoscopic volumes in unrestrained animals, our miniscope enables high-throughput investigation of spatially distributed circuits such as grid cells, head-direction cells, and border-cells ensembles in medial entorhinal cortex (Obenhaus et al., 2022), and generalizes systems-level studies aimed at uncovering the computational principles of natural behavior and high-level cognitive functions.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Title: Entorhinal ultraslow oscillations and sequences across behavioral conditions

Authors: *J. S. FELDMAN, L. BRAUN, A. LAUTRUP, E. I. MOSER, M.-B. MOSER, S. GONZALO COGNO;
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Abstract: The medial entorhinal cortex (MEC) hosts many of the brain's circuit elements for spatial navigation and episodic memory, operations that require neural activity to be organized across long durations of experience. We previously found that when mice were head-fixed on a wheel and ran in darkness, MEC cells exhibited ultraslow oscillations (USOs) (frequency < 0.1 Hz) where neural population activity was organized into periodic sequences [1]. It is yet unknown whether USOs and sequences are also present when the animal engages in more naturalistic behaviors. To address this question, we used Neuropixels probes to record from the MEC of mice under three different conditions: running on a wheel in darkness, as in [1], running in an open field arena in the presence of distal landmarks, and sleep. We found ultraslow sequences in Neuropixels recordings both when the animals were running on the wheel and in an open field arena. However, the sequential activity was characterized by interruptions, suggesting that the sequences are interleaved with non-sequential activity bouts, in which the neural population activity reorganizes. We found much variability in the duration of the sequences, yet all sequences were in the tens of seconds time scale. The variability in sequence duration does not seem to be explained by changes in the animal's speed or head direction. Moreover, we found that within one session, the MEC always generates the same sequence ordering, and that this ordering recurs over time. Finally, we found that ultraslow periodic activity persisted during sleep, particularly during slow wave sleep (SWS), and spanned across up and down states in the neural population activity. However, this ultraslow activity manifested only as ultraslow oscillations that were synchronous across the population. Repeating ultraslow sequences were not detected. USOs also manifested in the hippocampus and were often highly synchronous with those in the MEC. All in all, our results suggest the presence of internal dynamics that unfold at ultraslow time scales, and that are modulated by the state and behavior of the animal.
1. Gonzalo Cogno, S., et al., *Minute-scale oscillatory sequences in medial entorhinal cortex.* Nature, 2024. **625**(7994): p. 338-344.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Title: A bihemispheric vector computation mechanism for directional alternation of theta sweeps in grid cells

Authors: A. VOLLAN[1], ***R. SAXENA**[2], R. GARDNER[3], M. SCHELLENBERGER[4], M.-B. MOSER[5], E. I. MOSER[5];
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Abstract: Navigation relies on the brain's ability to flexibly represent not only the current position but also nearby and upcoming locations. Hippocampal place cells and entorhinal grid cells may support such look-ahead to the surrounding space, by expressing spatial trajectories that 'sweep' outward, in a left-right alternating pattern from the animal's current location within each theta cycle (Vollan et al., 2025). These sweeps are aligned with, and potentially steered by, an allocentric 'internal direction' (ID) signal encoded by cells in parasubiculum (PaS). Across successive theta cycles, the ID signal typically alternates between pointing to the egocentric left and right of the animal's head axis, without a consistent external correlate (thus internal). This alternation is absent in classical head direction (HD) signals in upstream regions projecting to PaS, which are tightly coupled to the animal's HD. This raises the question of whether and how upstream HD signals are transformed into ID signals within parahippocampal circuits. Experimental studies in Drosophila (Lyu et al., 2022) have characterized a neuronal circuit that performs vector addition of allocentric HD and egocentric traveling direction to compute allocentric traveling direction. A similar computation could be performed by conjunctive HDxID cells, tuned to both allocentric HD and ID, to convert the HD signal into ID by adding an egocentric directional component to it. Using high-density Neuropixels recordings in rats during open-field foraging, we identified such HDxID cells in PaS. These neurons show strong hemispheric lateralization: left hemisphere cells show a rightward phase shift in ID tuning relative to HD and fire on rightward sweeps, while right hemisphere cells show a leftward shift and fire on leftward sweeps. HDxID cells are located dorsally to pure ID cells and fire earlier in the theta cycle. Cross-correlogram analysis reveals directional connectivity from HDxID to ID cells. Based on the hemispheric asymmetry and directional connectivity, we propose that HDxID cells in the left and right hemispheres may act as a coupled oscillator, selectively biasing rightward and leftward theta sweeps, respectively, via downstream ID and conjunctive grid cells. This is reminiscent of central pattern generators observed in other oscillating brain systems involved in locomotion, respiration, etc. (Grillner, 2006; Marder & Buchner, 2001; Feldman et al. 2013).
These results provide a critical link in the circuit-level transformation from HD to ID, opening a window to further understand how HD inputs influence the generation of spatial maps in the hippocampal-entorhinal circuits.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Title: Goal-directed modulation of theta sweep patterns

Authors: *M. SCHELLENBERGER, A. VOLLAN, R. SAXENA, M.-B. MOSER, E. I. MOSER;
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Abstract: The hippocampal formation is thought to support memory and navigation by forming a "cognitive map" (O´Keefe & Nadel, 1978). For this map to be useful in spatial navigation, the external environment needs to be linked to the internal representation on a fast timescale. Such fast timescale links have been described for hippocampal place cells and entorhinal grid cells, which fire in sequences within individual ~125ms theta oscillation cycles, encoding trajectories that originate near the animal's current location and sweep outwards into ambient space (Vollan et al., Nature 2025). These theta sweeps in place cells and grid cells are aligned with an 'internal direction' signal encoded by cells in the parasubiculum. During free exploration and running on a linear track, consecutive theta sweeps are rigidly directed forwards towards alternating sides of the animal, with ~30-degree offsets from the head axis. While this is an efficient strategy for maximizing spatial coverage of the entire environment, directing theta sweeps towards regions of interest may be more beneficial during navigation to specific target locations.
By decoding population activity in the hippocampal formation using Neuropixels probes while rats perform multiple tasks, we find that theta sweep patterns can deviate from the stereotypical head-centered alternation when animals orient towards salient cues or rewards, or when they move in other directions than forwards. Our data shows that the average direction of theta sweeps (the sweep axis) can track a moving piece of bait during chasing behavior and that it points backwards when animals back out of a narrow corridor. Left-right alternation around the sweep axis can still be observed under these conditions. Internal direction signals in the parasubiculum are aligned with the deviations in the sweep axis of the grid cells. 'Classical' head direction cells in the anterior thalamus and presubiculum maintain their tuning to allocentric head direction. A similar split between internal direction and head-direction signals can occasionally be observed during REM sleep.

Taken together, these findings show that while rigid left-right alternation is the standard pattern of theta sweeps, sweeps can momentarily be directed towards salient locations in the environment. Dynamic modulation of the theta sweep pattern may provide a mechanism for targeted updating of the cognitive map during goal-directed navigation.

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Poster

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Title: Enhanced population drift in the hippocampal formation when position is clamped

Authors: *A. LAUTRUP, E. R. SKYTØEN, J. FELDMAN, E. I. MOSER, M.-B. MOSER, S. GONZALO COGNO;
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Abstract: Storage and retrieval of episodic memory in the hippocampus rely on both spatial and temporal elements of experience. The passage of time and experience are mirrored in the drifting activity of neural populations in the lateral entorhinal cortex (LEC). When animals are engaged in free foraging or during trial-based tasks, the population activity drifts at time scales from seconds to minutes and hours. Less drift has been reported in the medial entorhinal cortex (MEC) and hippocampus at these time scales. Whether these differences are due to unique features in the LEC neurons and network, or whether conditions exist in which also hippocampal and MEC populations drift at similar rates, remains to be determined. To address this question, we implanted high-density Neuropixels probes for simultaneous recording in the hippocampal CA1 region and LEC or MEC in mice. In addition to free foraging behaviour, we recorded head-fixed animals while they ran at a constant location on a rotating wheel in darkness and with no scheduled rewards. In the open field, we found less drift in MEC and CA1 than LEC, as expected. In contrast, on the wheel we found that the population activity in all regions travelled

further in neural state space, the neural trajectories were less folded over time, and time could be decoded with higher accuracy at the minute time scale. Spatially informative units, abundantly found in MEC and CA1, attenuate the drift in the open field. However, only a small proportion of units appeared to track spatial variables on the wheel, such as absolute position or distance run. Overall, our results suggest that neural population activity not only in LEC, but also in MEC and CA1, can drift at slow time scales, but that drift of hippocampal and MEC activity is attenuated in conditions where spatial landmarks are relevant for navigation.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Title: Encoding of location and navigational trajectories in retrosplenial and hippocampal cell assemblies

Authors: *H. ENEQVIST, J. CARPENTER, E. I. MOSER, M.-B. MOSER;
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Abstract: How is the cortex involved in navigation? Due to its anatomy and connectivity, the retrosplenial cortex (RSC) has recently gained a lot of attention as a gateway between the hippocampus and the neocortex. We aim to understand interactions between these regions further by investigating, with high-density Neuropixels 2.0 recordings, how neural population activity differs in the two regions as animals construct a cognitive map of space.
We recorded thousands of simultaneously active cells in the granular and agranular retrosplenial cortex and the CA1 region of the hippocampus (hundreds in each region). The implants were inserted perpendicular to the midline and spanned a range of retrosplenial coordinates. Due to its shape, all layers of the agranular region were recorded but in the granular region upper layers (2/3) were prioritized. We used two behavioral paradigms: exploration in an open field (80 x 80 cm) and goal-directed navigation in a Figure 8 maze, where alternation at the T junction was

rewarded.
Many RSC neurons were tuned to specific locations in the open field. We observed cells with border and corner fields, as well as head direction (HD) cells, in both regions and cells with firing fields away from the walls in the granular RSC. RSC cells developed and changed their spatial tuning alongside cells in CA1. The spatial information expressed by single cells in both regions in a new environment increased slowly within and across days. When animals moved between environments, the RSC exhibited signs of hippocampal-like remapping alongside global remapping in CA1. During remapping, RSC cells might change their functional spatial identity, e.g. from border to corner tuning. Egocentrically tuned cells rotated coherently with HD cells. Figure 8 maze pilot data showed that the RSC also encodes more complex task structures at both single cell and population levels. Individual cells expressed spatial activity similar to that in the open field, as well as task specific features, such as encoding of choice and reward points. Dimensionality reduction revealed that retrosplenial activity both represents location in the maze and can delineate between upcoming left and right turns as well as erroneous trials and changes in learning rules. We are currently exploring how position, trial-context and reward are affecting the population activity and how it differs from what can be seen in the hippocampus. Understanding the contributions of retrosplenial dynamics to the coding of space will help disentangle how spatial information is processed in the cortex in tandem with the hippocampus.

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Poster

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Title: Developmental Trajectories of Functional Cell Types in the Medial Entorhinal Cortex

Authors: *H. A. OBENHAUS[1], H. ENEQVIST[2], T. SLETTMOEN[1], R. JACOBSEN[1], M. POFAHL[2], F. DONATO[3], M.-B. MOSER[1], E. I. MOSER[1];
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Abstract: Spatial navigation relies on the precise coordination of neuronal networks composed of specialized cells that collectively encode the surrounding environment, many of which have been identified in the adult mammalian medial entorhinal cortex (MEC) and the adjacent parasubiculum (PAS). While the fundamental components of this system have been extensively characterized following the completion of development, it remains unclear how functional specificity emerges during the earliest stages of cortical formation - when neurons are still migrating and integrating into networks within the MEC. In particular, the relationship between anatomical developmental gradients (Donato et al., 2017) and the emergence of functional specificity in the MEC and PAS has yet to be elucidated.
To investigate this question, we performed single, ultrasound-guided viral injections of Cre-recombinase into the ventricles of GCaMP transgenic embryos at embryonic days E12 to E15. This approach enabled sparse labeling of post-mitotic cells migrating to the cortex within narrow developmental time windows. In adulthood, the same mice received local injections of a floxed tdTomato virus into the MEC and PAS, allowing us to visualize these cells using two-photon head-mounted miniscopes (MINI2P) in freely moving animals. This technique enabled us to image isochronic cells - those born at similar embryonic timepoints - and compare their functional properties to neighboring cells of unspecified temporal origin in the same animal. Our preliminary results indicate that grid cells undergo a distinct developmental progression, with cells from the most frequently observed medium spaced module (M2) showing an enrichment in isochronic populations labeled around embryonic days E13 and E14. In contrast, the fraction of head direction-modulated cells appears reduced during this same developmental window, indicating that different spatial cell types may arise from distinct temporal time windows during development. We are currently investigating whether similar developmental patterns are present in other spatially tuned cell types, such as border cells and object vector cells, and whether functional identity across these populations can be linked to the two major principal cell classes in the MEC - stellate and pyramidal cells - across developmental timepoints.

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Poster

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Title: Does the medial entorhinal cortex provide different functional input to the CA3 and dentate gyrus subfields of the hippocampus?

Authors: *R. JACOBSEN, H. A. OBENHAUS, M.-B. MOSER, E. I. MOSER;
Kavli Inst. for Systems Neurosci., Trondheim, Norway

Abstract: The brain encodes position within an environment using spatially selective cells. These include place cells in the hippocampus whose location-specific firing patterns are termed place fields, and several functional cell types such as grid and head direction cells in the medial entorhinal cortex (MEC). These areas are well connected; layer 2 of the MEC sends direct projections to both the CA3 and dentate gyrus (DG) subfields of the hippocampus. However, the properties of the principal cells in these two subfields are markedly different. Most pyramidal cells in CA3 typically display one place field and are thought to contribute to pattern completion, while granule cells in DG show sparse activity and very few have place fields in a given environment, in line with the concept of pattern separation. However, whether these functional differences are associated with distinct input populations of functional cell types in the MEC remains to be determined.
To determine if the information MEC sends to the hippocampus differs for CA3 and DG, we use transgenic mice and a modified rabies virus carrying GCaMP to target and label these subfields' monosynaptic inputs in the superficial layers of MEC. We then image these cells with a head-mounted 2-photon miniscope (MINI2P), which enables the identification of all known spatial cell types in the MEC while the animal is exploring an open field environment. With this method we are able to image functional cells in the MEC that provide direct, monosynaptic input to either CA3 or DG, including grid and aperiodic spatial cells.
We are currently investigating if the composition of the various functional cell types projecting to CA3 and DG differ on the population level. These results will help us understand whether the different spatial properties of cells in distinct hippocampal subfields may be inherited from their entorhinal input.

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Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Title: Functional independence of grid cell modules during hippocampal remapping

Authors: C. M. LYKKEN, ***B. R. KANTER**, A. NAGELHUS, J. CARPENTER, M. GUARDAMAGNA, E. I. MOSER, M.-B. MOSER;
NTNU, Trondheim, Norway

Abstract: Hippocampal place cells randomly reorganize their collective activity pattern (or 'remap') in response to environmental change, often resulting in orthogonal activity patterns for any pair of environments. The high dimensionality of the place code enables the network to store large numbers of patterns with minimal overlap, a fundamental requirement of an episodic memory system. This orthogonality is not present upstream in the medial entorhinal cortex (MEC), where the neural activity of grid and head direction cells is constrained to low-dimensional manifolds. It is still unclear how spatial maps are converted from a fixed set of states in the MEC to a seemingly vast number of uncorrelated representations in the hippocampus. Here, we used large-scale, multisite Neuropixels probes to simultaneously record from grid cells in three or more modules and hippocampal place cells. We provide the first experimental evidence that grid cells of different grid modules shift their spatial firing patterns independently between distinct, familiar environments, and that this functional independence coincides with full orthogonalization ('global remapping') of downstream place cells. In experiments where one pair of grid modules maintained coherence, the orthogonalization of the place code was incomplete ('partial remapping'). The degree of module independence was the strongest predictor of the strength of place cell remapping. In contrast to the independent changes in module phase, grid modules rotated coherently under all experimental conditions. Importantly, we were able to reproduce each of these experimental results using network simulations of the grid-to-place cell transformation. A simple model connecting idealized modules of grid cells to downstream place cells captured the whole continuum of remapping results observed in our experiments. Together, these results point to the modularization of grid cells as a mechanism underlying orthogonalization of spatial maps and high-capacity memory storage in the hippocampus.

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Poster

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212262/Z/18/Z
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Title: Spatial uncertainty determines how navigation and grid cell tuning are shaped by environmental geometry

Authors: *Y. H. R. KANG[1], D. M. WOLPERT[2], M. LENGYEL[3];
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Abstract: Variations in environmental geometry, such as the shape and size of an enclosure, have profound effects on navigational behavior and its neural underpinning in grid cells. Here, we show that these effects arise as a consequence of a single, unifying principle: to navigate efficiently, the brain must maintain and update the uncertainty about one's location. We developed an image-computable Bayesian ideal observer model of navigation, continually combining noisy visual and self-motion inputs, and a neural encoding model representing the spatial uncertainty computed by the ideal observer. Through mathematical analysis and numerical simulations, we show that the ideal observer's spatial uncertainty accounts for a diverse range of sometimes paradoxical distortions of human homing behavior in anisotropic and deformed environments. Moreover, the neural encoding of this uncertainty accounts for distortions of grid cell responses under identical environmental manipulations. Our results suggest that spatial uncertainty plays a fundamental role in navigation.

Disclosures: Y.H.R. Kang: None. **D.M. Wolpert:** None. **M. Lengyel:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.16/QQ16

Topic: I.09. Spatial Navigation

Support: NIH/NINDS Intramural Research Program

Title: The role of astrocytes in the medial entorhinal cortex during spatial learning

Authors: *K. ZHANG, Y. MA, T. MALONE, Y. GU;
NINDS, Natl. Inst. of health, Bethesda, MD

Abstract: Astrocytes are widely distributed throughout the brain, where they support neuronal functions through regulation of synaptic transmission, metabolism, and neurotrophic production.

Astrocytes are also abundant in the medial entorhinal cortex (MEC), which plays a critical role in spatial cognition. Although MEC dysfunction is known to impair spatial learning and memory, and contributes to spatial cognition decline in Alzheimer's disease, the specific role of MEC astrocytes in spatial memory remains unclear. In this study, we combined *in vivo* two-photon calcium imaging with virtual reality (VR) navigation task to investigate astrocytic activity in layer 2 of the mouse MEC during spatial learning. Water-restricted mice unidirectionally navigated in a novel VR environment, which was a 10-meter-long linear track with spatial landmarks and a water reward at fixed locations. We observed that astrocyte calcium dynamics, which were measured by the fluorescent intensity of genetically encoded calcium indicator GCaMP6f, did not show spatial activity pattern during navigation. Instead, their activity was primarily modulated by locomotion onset, independent of reward delivery or environmental familiarity. These results suggest that astrocytes are involved in speed modulation of MEC neural dynamics. Chemogenetic inhibition of neural activity did not suppress astrocytic calcium response but did accelerate their rise kinetics. Notably, inhibiting astrocytic calcium activity disrupted the speed modulation of neuronal firing, reduced stability and selectivity of spatial representation by neurons, and impaired spatial learning performance. These findings suggest that while MEC astrocytes do not encode spatial information directly, they play a critical modulatory role in regulating the speed-dependent dynamics of neuronal activity, thereby influencing spatial representation by neurons and spatial learning. Our study highlights a novel contribution of astrocyte to the neural mechanisms underlying spatial cognition.

Disclosures: K. Zhang: None. **Y. Ma:** None. **T. Malone:** None. **Y. Gu:** None.

Poster

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.17/QQ17

Topic: I.09. Spatial Navigation

Support: NIH/NINDS Intramural Research Program

Title: Inflexible spatial encoding prevents the stabilization of the entorhinal cognitive map in tauopathy mice

Authors: *T. MALONE[1,2], K. CEKADA[2], J. TYAN[2], G. WANG[2], Y. GU[3];
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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.

Disclosures: T. Malone: None. **K. Cekada:** None. **J. Tyan:** None. **G. Wang:** None. **Y. Gu:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.18/QQ18

Topic: I.09. Spatial Navigation

Support: NINDS

Title: Hallmarks of slow synaptic plasticity in gradual mapping of novel spaces by grid cells

Authors: *L. CHEN[1], L. DONG[3], F. SHAHID[6], T. MALONE[7], N.-W. TIEN[2], K. CEKADA[8], S. CHANDRA[4], I. R. FIETE[5], Y. GU[9];
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Disorders and Stroke, Rockville, MD; [8]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [9]NIH, Bethesda, MD

Abstract: Animals construct internal "cognitive maps" of the world during navigation in spatial and non-spatial domains, with grid cells in the medial entorhinal cortex (MEC) playing a key role. This requires associating internal position estimates with external cues to reduce spatial uncertainty over time. However, how grid cell representations evolve in novel spaces to support map formation is unclear. To address this question, we longitudinally record grid cells with two-photon calcium imaging over 10 days as animals learn operant tasks in novel virtual linear tracks. We observe that spatial tuning of grid cells is present immediately but evolves as a significant fraction of spatial fields shift backward on a run-by-run basis, within and across days. Backward shifts are more prevalent and persistent in successful learners. The fields gradually stabilize across days, anchored by those near landmarks, suggesting a slow plasticity mechanism. The backward shifts partially reset daily, reflecting a second slow timescale of plasticity. While individual fields of a cell shift differentially, co-active fields of co-modular grid cells shift together, indicating their coupled dynamics that remain on the same two-dimensional torus. Spatial learning leads to systematic changes and stabilization of the population phase trajectory, including lateral shifting, rotation, and phase resetting at landmarks. These findings align with a model involving temporally delayed Hebbian plasticity from cue-driven hippocampal cells to grid cells, enhancing the fidelity of the spatial map. Our study shows how slow plasticity gradually establishes a stable coexistence of internal and cue-driven representations, elucidating cognitive map formation.

Disclosures: L. chen: None. **L. Dong:** None. **F. Shahid:** None. **T. Malone:** None. **N. Tien:** None. **K. Cekada:** None. **S. Chandra:** None. **I.R. Fiete:** None. **Y. Gu:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.19/QQ19

Topic: I.09. Spatial Navigation

Support: NIH/NINDS Intramural Research Program

Title: The entorhinal spatial map integrates visual identity information of landmarks

Authors: G. WANG[1,2], ***F. SHAHID**[1], T. MALONE[1], J. TYAN[1,3], K. CEKADA[1], Y. GU[1]; [1]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [2]Med. Scientist Training Program, Med. Col. of Wisconsin, Milwaukee, WI; [3]Natl. Inst. of Diabetes, Digestive, and Kidney Dis., Bethesda, MD

Abstract: The location and identity of landmarks provide spatial and nonspatial information of an environment, respectively, to guide spatial navigation. While the medial entorhinal cortex (MEC) is essential for representing spatial information, it is unclear whether it also encodes

landmark identity. Here, we conducted two-photon calcium imaging of the MEC when mice navigated in multiple virtual environments, and discovered a general ability of the MEC to encode landmark identity through cue cells, which responded to individual landmarks during virtual navigation. Cue cells represented landmark identity by exhibiting more distinct activity patterns between visually disparate landmarks than identical ones. The representation was modulated by the spatial shift of cue cell activity relative to landmark location and was independent of landmark frequency and proximity to a water reward. Moreover, the identity encoding by the same cue cell population changed between different environments but was maintained within the same environment despite increased experience. In contrast, landmark location encoding by cue cells was regulated by experience, suggesting different mechanisms underlying the encodings of landmark identity and location. Finally, compared to cue cells, grid cells weakly encoded landmark identity but more robustly encoded landmark location. Thus, the MEC integrates both spatial and nonspatial landmark identity information during navigation, but potentially through different circuit mechanisms.

Disclosures: G. Wang: None. **F. Shahid:** None. **T. Malone:** None. **J. Tyan:** None. **K. Cekada:** None. **Y. Gu:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR415.20/QQ20

Topic: I.09. Spatial Navigation

Title: Differential long-term dynamics of entorhinal grid and spatial cells

Authors: S. WEN[1], ***X. YUAN**[1], C. MIAO[1], M. R. MEHTA[2];
[1]Peking Univ., Beijing, China; [2]Departments of: Physics & Astronomy, Neurology, Neurobio., Univ. of California at Los Angeles (UCLA), Los Angeles, CA

Abstract: The entorhinal-hippocampal circuit is crucial for spatial learning via synaptic plasticity. Consistently, hippocampal place cells and entorhinal grid cells show rapid, experience-dependent systematic changes, i.e. functional plasticity, on one-dimensional tracks. However, their long-term dynamics with repeated exposures to an environment, and its nature during unconstrained two-dimensional maze exploration, are unknown. Paradoxically, recent studies show long-term random drift, not systematic changes, of hippocampal place fields during random foraging. To address these mysteries, we measured the activity of many neurons in the medial entorhinal cortex (MEC) and from dorsal CA1, of freely behaving mice over one month using advanced calcium imaging. In addition to random-drift, grid and spatial cells' receptive fields showed long-term systematic shift, a phenomenon not previously reported. There were major differences in the long-term shifts of MEC grid and spatial cells -individual grid cells' ratemaps changed by a small amount but the changes were coherent across the population, which is consistent with the attractor hypothesis; whereas simultaneously recorded individual spatial

cells' ratemaps changed by a larger amount but the changes were not coherent across the population. As a result, the long-term shift of simultaneously recorded grid cells formed a vector field that showed a net divergence and gradient whereas spatial cells only showed significant divergence but not gradient. Further, CA1 place cells did not show any systematic shift and its random drift was significantly less than that in entorhinal grid and spatial cells. These results provide the first evidence of long-term functional plasticity of entorhinal grid and spatial cells in two dimensions. Although grid and spatial cells are anatomically intermixed, their differential dynamics suggests potential decoupling of these networks. Further, the large differences between entorhinal versus hippocampal long-term dynamics indicates crucial contribution of additional mechanisms governing entorhinal to hippocampal coupling.

Disclosures: S. Wen: None. **X. Yuan:** None. **C. miao:** None. **M.R. Mehta:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR415.21/RR1

Topic: I.09. Spatial Navigation

Title: The role of the CA1 pyramidal neuron specific micropeptide CTXND1 in Alzheimer's disease

Authors: *Y. LI, C. MIAO;
Peking Univ., Beijing, China

Abstract: The hippocampus plays a crucial role in spatial memory, but the underlying molecular mechanisms require further exploration. In this study, we identified *Ctxnd1* as a gene specifically expressed in the hippocampal CA1 pyramidal neuron with its decreased expression in Alzheimer's disease in both mice and human. Both *in vitro* overexpression assays and *in vivo* detection by polyclonal antibody demonstrated that *Ctxnd1* encodes a functional micropeptide, which is 59 amino acids long and is highly conserved in primates. Overexpression of *Ctxnd1* in 5xFAD mice enhances their performance in both the Y-maze and Morris water maze tests. Additionally, we observed a significant reduction in Aβ plaque density in the brains of *Ctxnd1*-overexpressing 5xFAD mice. In summary, this study suggests that the gene *Ctxnd1*, specifically expressed in the CA1 region of the hippocampus, may play an important role in hippocampal related spatial memory in Alzheimer's disease.

Disclosures: Y. Li: None. **C. miao:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

Location: SDCC Halls B-H

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Program #/Poster #: PSTR415.22/RR2

Topic: I.09. Spatial Navigation

Title: Directional dynamics in the entorhinal cortex driven by behavioral constraints

Authors: *R. LIU;
Peking Univ., Beijing, China

Abstract: The medial entorhinal cortex of rodents contains a variety of functionally-defined cell types, including grid and head direction cells. By comparing neural recordings in mice exploring the same environment either freely or head-fixed to an autonomous-driving cart, we here show that, for a substantial portion of neurons, the head direction functional identity is dynamic, likely emerging through self-organization. Approximately half of free-foraging head direction cells reversibly lost their tuning during assisted navigation, while other neurons gained directional tuning, especially in environments enriched with sensory cues. This adaptive process is experience-dependent, progressively sharpening and stabilizing directional representations, while also integrating a spatial component. Our findings reveal a remarkable flexibility in entorhinal circuits, demonstrating their capacity to reorganize and adapt based on how animals interact with their environment.

Disclosures: R. Liu: None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR415.23/RR3

Topic: I.09. Spatial Navigation

Title: Mice dCA1 responds to abstract videos with progress-related contextual codes

Authors: *G. KAI, C. MIAO;
Peking Univ., Beijing, China

Abstract: The hippocampus in non-human mammals has been established as critical for both spatial navigation and episodic-like memory, consistent with its role in cognitive mapping and experience-dependent memory. One building block of episodic memory is visual features in space and time, but experimental evidences of how hippocampal mnemonic framework generally supports their integration remains insufficient. In this study, video stimulus, by presenting 20 videos and 14 repeats each in a random order, were providing organized visual sequences to test memory encoding in mice. To retain visual continuity and remove spatial components, videos are sampled from continuous trajectories in latent space of auto-encoders. Two different behavioral paradigms are involved. For active manipulation task (AMT), the playback speed is

controlled by running speed of mice on a spherical treadmill. For passive viewing task (PVT), all videos are played at a constant speed of either 150 fps or 240 fps. Results show that in both cases, CA1 neurons formulate movie fields, allowing discrimination of video identity and progress. Response features are compared among videos, tasks and playback speeds. These responses provide a basic framework for memorization and discrimination of spatial-temporal organization of visual features in episodic experiences.

Disclosures: G. Kai: None. **C. miao:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR415.24/RR4

Topic: I.09. Spatial Navigation

Title: The representation of social spatial information in the medial entorhinal cortex

Authors: *Q. XU, H. CAI, C. MIAO;
Peking university, Beijing, China

Abstract: As social animals, mice rely heavily on social interactions for survival and development, which necessitates the real-time representation of both self and conspecific positions during social encounters. The medial entorhinal cortex (MEC) plays a critical role in spatial memory and path integration, housing diverse cell types that encode self-location information, such as grid cells and border cells. While previous studies have identified cells representing the positions of conspecifics in hippocampal CA1, it remains unclear whether the MEC, a key upstream input region of the hippocampus, also encodes social spatial information. In this study, we employed two-photon calcium imaging in freely moving mice during social interactions and identified three distinct cell types in the MEC that encode social spatial information: other spatial cells, egocentric social-spatial cells, and allocentric social-spatial cells. Upon environmental changes, both egocentric and allocentric social-spatial cells exhibited remapping, with egocentric social-spatial cells demonstrating greater sensitivity to environmental scale. In addition, these cell types differentially encoded social partners of varying familiarity, exhibiting higher precision in representing unfamiliar conspecifics. Our findings suggest that the MEC plays a pivotal role in the neural encoding of social spatial information.

Disclosures: Q. Xu: None. **H. Cai:** None. **C. miao:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Topic: I.09. Spatial Navigation

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Qidong-SLS Innovation Fund (Grant No. 2023002029 and 2020001540)

Title: Bglap3: Age-related Molecular Regulator of Neuronal Excitability and Spatial Coding in the Medial Entorhinal Cortex

Authors: *S. XU[1,2,3], S. UZEEN[2,1], Z. HUANG[4,2], C. MIAO[3,2,1];
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Abstract: Age-related cognitive decline, particularly in spatial navigation, is associated with dysfunction of the medial entorhinal cortex (MEC), a critical structure for spatial representation and memory formation. However, the molecular mechanisms underlying age-dependent MEC dysfunction remain poorly understood. Here, we identified Bglap3, a gene with specifically enriched in MEC layer III, that exhibits significant downregulation in aged mice (>22 months) compared to young mice (3-4 months). Transcriptomic analysis following Bglap3 manipulation revealed differential regulation of trans-synaptic signaling and neuronal excitability, suggesting its involvement in neuronal communication. Using targeted knockdown approaches (RNAi) in young mice, we demonstrated that knock down of Bglap3 significantly decreased the intrinsic excitability of MEC layer II stellate cells, reduced the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs) and diminished dendritic spine density. In vivo two-photon calcium imaging revealed that Bglap3 knockdown disrupted the functional integrity of MEC spatially-tuned neurons, including grid cells, head-direction cells, and border cells. These cellular and circuit alterations were accompanied by impaired performance in both standard Morris water maze tasks and path-integration-dependent navigation. Finally, we identified GPR158 as a potential receptor of Bglap3. Our findings establish Bglap3 as a critical molecular regulator of MEC neuronal function that specifically changed in the aging MEC.

Disclosures: S. Xu: None. **S. Uzeen:** None. **Z. Huang:** None. **C. miao:** None.

Poster

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Program #/Poster #: PSTR415.26/RR6

Topic: I.09. Spatial Navigation

Title: Multisensory deprivation disrupt spatial-coding in the medial entorhinal cortex

Authors: *J. TIAN[1], S. WEN[1], Y. ZHOU[1], S. YAO[1], X. ZHANG[2], C. MIAO[1,2,3,4];
[1]Sch. of Life Sci., [2]Peking-Tsinghua Ctr. for Life Sci., [3]PKU-IDG/McGovern Inst. for Brain

Res., Peking Univ., Beijing, China; [4]Chinese Inst. for Brain Research, Beijing (CIBR), Beijing, China

Abstract: Spatial navigation relies on integrating self-motion cues with external sensory inputs. The medial entorhinal cortex (MEC) hosts diverse spatially modulated neurons—head direction (HD), border, grid, and speed cells—that contribute to spatial representation. While visual influences on MEC activity are well-characterized, the role of tactile inputs, particularly whisker-mediated signals, is less understood. We employed miniature two-photon calcium imaging to monitor neuronal activity in MEC of freely moving mice under sequential sensory deprivation: baseline (light), darkness, and darkness combined with whisker trimming. This experimental design enabled us to dissect the distinct contributions of visual and tactile inputs to spatial coding. From 3,758 recorded neurons across 13 mice, we identified spatially modulated neurons during the baseline light session. Comparative analyses across deprivation conditions revealed that HD and border cells exhibited significant disruption under both visual deprivation and whisker trimming. In contrast, grid cells and speed cells were primarily impaired by visual loss but remained relatively resilient to additional tactile deprivation. Notably, a subset of MEC neurons displayed activity correlated with whisker movement, and some responded selectively to textured surfaces. This suggests that mice may transmit tactile information via their whiskers to brain regions such as MEC, contributing to the maintenance of spatial representation and facilitating spatial localization. Collectively, our study highlights the distinct and complementary roles of visual and tactile inputs in modulating spatially tuned MEC neurons and underscores the heterogeneity in sensory integration strategies across cell types.

Disclosures: J. Tian: None. **S. Wen:** None. **Y. Zhou:** None. **S. Yao:** None. **X. zhang:** None. **C. Miao:** None.

Poster

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Program #/Poster #: PSTR415.27/RR7

Topic: I.09. Spatial Navigation

Title: Gpr6 Protects Medial Entorhinal Cortex Circuits from Amyloid Pathology in Alzheimer's Disease

Authors: *Z. WANG[1,2], X. ZHANG[2], C. MIAO[2];
[1]Acad. for Advanced Interdisciplinary Studies, Beijing, China; [2]Peking university, Beijing, China

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by memory loss and cognitive decline but molecular signatures of which are largely unknown. The medial entorhinal cortex (MEC) is thought to be one of the most relevant regions associated with spatial memory impairment in AD. Using 10X Visium spatial transcriptomics, we identified

Gpr6 as a specifically enriched gene in MEC layer III, whose expression significantly declined in 5×FAD mouse model. Overexpression of Gpr6 in the MEC improved spatial memory in the Barnes maze, Morris water maze, and Y-maze, accompanied by reduction in Aβ deposition. Also, the number of microglia decreased, suggesting the inhibition of neuroinflammation. In vivo two-photon calcium imaging revealed that overexpressing Gpr6 in AD rectified MEC neuron spiking activity and enhanced context-dependent spatial coding. Consequently, these results demonstrate that Gpr6 plays a protective role in MEC circuit from amyloid pathology in AD, highlighting the therapeutic potential of Gpr6 for mitigating early MEC function deficits and preventing AD progression.

Disclosures: Z. Wang: None. **X. zhang:** None. **C. miao:** None.

Poster

PSTR415: Grid Cells and Spatially Modulated Cells

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Program #/Poster #: PSTR415.28/RR8

Topic: I.09. Spatial Navigation

Title: Spiking Tolman-Eichenbaum Machine: A computational model for place cells and grid cells

Authors: *D. KAWAHARA[1], S. FUJISAWA[2];
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Abstract: Place cells in the hippocampus exhibit location-specific firing to construct a cognitive map of the environment, while grid cells in the medial entorhinal cortex display a periodic hexagonal firing pattern that encodes self-motion and metric space. The coordinated activity of these two cell types underlies spatial navigation and memory in mammals.A key temporal feature of these cells is phase precession, wherein the spike timing of a neuron shifts to earlier phases of the theta oscillation as the animal traverses its place or grid field. Phase precession is thought to play a crucial role in temporal coding by compressing sequential experiences into single theta cycles, thereby supporting functions such as path planning, sequence learning, and prospective coding.The Tolman-Eichenbaum Machine (TEM) has been proposed as a computational framework that unifies the representation of spatial and relational memory and accounts for various firing patterns of place cells and grid cells. However, the standard TEM is a rate-based model trained using backpropagation, and as such, it does not incorporate spike timing dynamics that are essential for modeling phenomena such as phase precession and phase locking.To address this limitation, we propose the Spiking Tolman-Eichenbaum Machine (Spiking TEM)—a biologically plausible extension of the original rate-based model, implemented with spiking neurons. Our model not only reproduced the spatial firing patterns of place and grid cells in both linear track and two-dimensional open field environments but also reproduced the phenomenon of phase precession, wherein spike timing advances within the theta

cycle as the animal traverses a place or grid field.Moreover, by analyzing the learned synaptic weights of the network, we elucidated how specific upstream input patterns contribute to the emergence of the characteristic hexagonal firing fields of grid cells—a mechanism that has remained largely uncharacterized in previous models.Anatomically, grid cells in the medial entorhinal cortex exhibit a dorsoventral gradient in grid scale, with larger-scale grids located ventrally and finer-scale grids dorsally. While the original TEM assumes the existence of pre-defined modules along this axis, we revealed that such modular structures can emerge spontaneously through learning. This finding suggests that the grid modules may not need to be hardwired, but could instead arise from experience-dependent synaptic plasticity.

Disclosures: D. Kawahara: None. **S. Fujisawa:** None.

Poster

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Topic: I.09. Spatial Navigation

Support: NIH Grant R00NS116129
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Title: Grid maps are disrupted after large-scale expansion of the recording arena and partially recover with experience

Authors: *B. GUTIERREZ-GUZMAN[1], J. HERNANDEZ PEREZ[1], H. DANNENBERG[1,2]; [1]Bioengineering, [2]Interdisciplinary Program of Neurosci., George Mason Univ., Fairfax, VA

Abstract: The spatially periodic firing of grid cells in the medial entorhinal cortex (MEC) is believed to provide a universal spatial metric regardless of an environment's spatial scale. Previous evidence showed that the hexagonal firing pattern of grid cells (grid maps) scales in response to scaling of a familiar arena, i.e., both field size and grid spacing increase when a familiar arena becomes larger. However, whether there is a limit to the extent to which grid maps can scale in response to scaling of an arena, is unknown. By recording MEC grid cells in freely behaving mice that sequentially forage in a small and medium-scaled (1.33) or small and large-scaled (1.66) arenas, we demonstrate that spatial periodicity in grid cell firing is substantially disrupted in the large-scaled but not the medium-scaled arena. Intriguingly, grid maps were disrupted when the scaling of the environment was larger than the scale ratio of successive grid cell modules. Next, we tested whether this disruption was permanent or transient. We observed that grid patterns re-emerged in the large-scaled arena after repeated experiences of transitioning from a small to the large-scaled arena. Taken together, these results show that grid maps are not universal across all environments and suggest that grid cells must learn to represent large-scaled spaces through an animal's experience.

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Poster

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Topic: I.09. Spatial Navigation

Support: NIH Grant NS053907

Title: The Origin of the Grid Cell Signal

Authors: *Z. HAGBI, J. S. TAUBE;
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Abstract: Wayfinding and orienting in an environment require forming an internal cognitive map in the brain. This map is formed by several types of neurons that can be found in different brain areas and encoding various aspects of spatial orientation. Grid cells are an example of spatial neurons which encode and integrate information about location, distance, and direction. Grid cells were first identified in the Medial Entorhinal Cortex (MEC), but have since also been identified in the Presubiculum (PrS) and Parasubiculum (PaS). While it has long been believed that the grid-cell signal originates in the MEC, this hypothesis remains unproven. Because both the PrS and PaS project to layers II and III of the MEC, where the highest percentage of grid cells have been found, this situation raises the possibility that these upstream areas may generate the grid signal, which is then projected to the MEC. Here, we tested this hypothesis by bilaterally inhibiting the MEC using chemogenetic DREADD's and its ligand CNO, while simultaneously recording grid cell activity in PrS or PaS. Our preliminary data of grid cell recordings in the PrS while inhibiting the MEC has exhibited two different patterns. One grid cell continued to fire in a hexagonal grid pattern, but the pattern was distorted, and the grid signal was not as robust following CNO injection. This response recovered after two hours of CNO injection. In contrast, another grid cell responded with complete disruption of the grid pattern following CNO application. However, this cell never recovered its grid pattern activity after two hours. These ambiguous results require further investigation to determine whether the origin of the grid signal is in MEC or in either the PrS or PaS.

Disclosures: Z. Hagbi: None. **J.S. Taube:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.01/RR11

Topic: I.10. Human Learning and Cognition

Support: UNAM-PAPIIT (IN207325)

Title: The primate hippocampus represents time and task features in a metronome task

Authors: *A. M. MALAGÓN[1,2], M. SALGADO-MENEZ[1], V. DE LAFUENTE[1];
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Abstract: Neural activity encoding temporal information has been found across cortical and subcortical structures. Recently, the hippocampus of rodents, primates, and humans has been shown to represent elapsed time in the context of spatial or episodic memory tasks. However, it's not clear whether the primate hippocampus represents time through temporal cells defined as "time cells." Furthermore, the precise mechanisms by which the hippocampus represents time in tasks where temporal perception is essential for successful behaviors remain largely unknown. To address these questions, we recorded the activity of single neurons in the hippocampus of two rhesus monkeys performing a visual metronome task. In this task, subjects track and internally maintain a temporal rhythm, defined by a visual stimulus that changes periodically from left to right across three different tempos, in the absence of external cues. Our findings reveal that distinct groups of hippocampal neurons represent key features of the task. Specifically, one group of neurons represents elapsed time in a manner that's either increasing or decreasing across trials, different from typical “time cells”. Additionally, hippocampal neurons encode the specific tempo of each trial and indicate whether the metronome stimulus was visible or not, reflecting both perception and internal maintenance phases of the task.

Disclosures: A.M. Malagón: None. **M. Salgado-Menez:** None. **V. de Lafuente:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.02/RR12

Topic: I.10. Human Learning and Cognition

Support: Hyogo Innovative Challenge, Hyogo Medical University
Hyogo Medical University Grant for Research Promotion
Cooperative Study Program of National Institute for Physiological Sciences

Title: Internal context in memory: Respiratory-phase alignment between encoding and retrieval modulates cognitive load in humans

Authors: *N. H. NAKAMURA[1], K. YOSHINO[2], M. FUKUNAGA[3];
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Abstract: Memory formation relies on two key elements of information: items and contexts. Although contexts involve not only external environments but also internal states of viscera, whether such as breathing phases act context in memory remains unclear. In our previous study using genetically modified mice and optogenetics, respiration during encoding were directly manipulated by the activation of the primary inspiratory rhythm generator PreBötzinger complex in the brainstem. The intervention approach showed that the timing of respiration during encoding is crucial for hippocampal memory formation and subsequent memory performance (Nakamura et al., nat commun 14:4391, 2023). To determine whether respiratory phases function as reinstatable context between encoding and retrieval, we employed 30 healthy human volunteers performing a delayed matching-to-sample recognition memory task, while their breathing waveforms were monitored. The present study has brought that a combination of respiratory phase matching between encoding and retrieval can increase and decrease the reaction time and cognitive load in the task. We will discuss about a further possible correspondence between respiratory phases and cognitive load.

Disclosures: N.H. Nakamura: None. **K. Yoshino:** None. **M. Fukunaga:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.03/RR13

Topic: I.10. Human Learning and Cognition

Title: Human hippocampal dynamics during naturalistic viewing and memory recall

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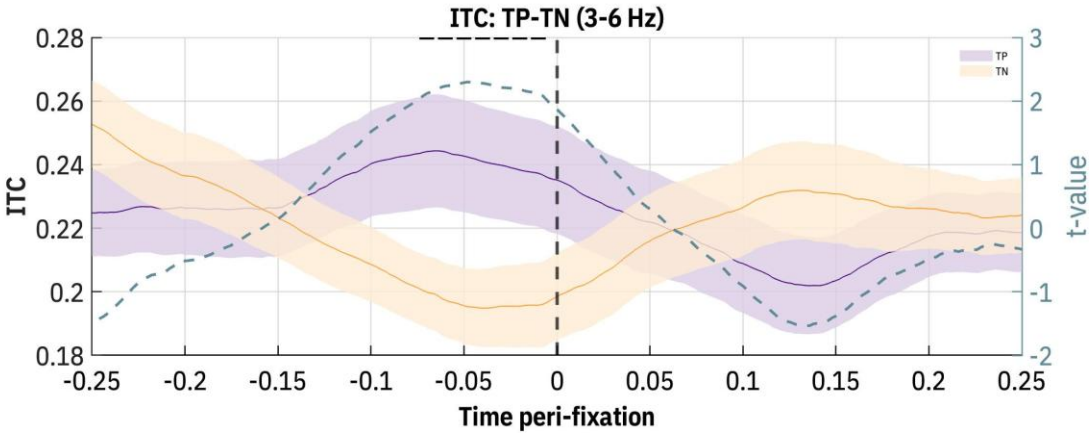
Abstract: Hippocampal theta rhythms synchronise before saccades to familiar spatial targets learned in controlled memory tasks, reflecting memory-guided visual cognition (Kragel et al., 2020). Whether this phenomenon also occurs during the exploration of stimuli that were encoded during naturalistic viewing remains unknown, but if true, it would underscore its synchronisation's ecological relevance. To answer this question, we analysed an open-access dataset (Keles et al., 2024) from 20 neurosurgical patients with intracranial electrodes (n = 63 hippocampal contacts) who observed an 8-minute movie clip (naturalistic encoding) and later completed a recognition test involving 20 old and 20 new movie frames. Recognition trials were binned in two conditions: correctly remembered (true positive, TP) and correctly identified as

new (true negative, TN). Fixation counts in the two conditions were matched via random subsampling (n = 16 per subject per condition), and their duration (mean TP = 0.47 s; TN = 0.44 s) and gaze position (mean TP=-1.11 rad; TN=-0.63 rad) were statistically indistinguishable (both p >0.28). Power spectral densities revealed theta-band (3 - 10 Hz) peaks in approximately 70% of hippocampal electrodes. To examine whether theta synchrony occurred around fixations, we computed fixation-locked inter-trial phase coherence (ITPC). After averaging the ITC within the 3-6 Hz range for each condition and performing a sliding-window paired t-test, we found that TP > TN ITC was significant from approximately -100 to 0 ms before a fixation occurred ($p < 0.05$, $t > 1.5$). Thus, a robust increase in hippocampal theta coherence preceded memory-related fixations, consistent with a role for hippocampal-visual coordination in the retrieval of naturalistic memories.



Disclosures: C. Castelblanco Riveros: None. **P.A. Angeli:** None. **K. Bujarski:** None. **C. Robertson:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.04/RR14

Topic: I.10. Human Learning and Cognition

Support: NIH Grant F30MH134507

Title: Human single-unit activity and coordination during categorical decision making

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Abstract: Our understanding of the meaning of words, images, and concepts relies on bidirectional signaling: integrating incoming perceptual information with memory-based

representations. The anterior temporal lobe (ATL) plays a central role in combining perceptual signals and memory-driven inputs to support recognition and categorization. We studied the neural dynamics underlying this integration using two recording modalities in epilepsy patients: intracranial EEG (iEEG; n = 26) and single-unit recordings via Utah arrays (n = 22). Participants performed a semantic categorization task, classifying images into four categories: animal, object, person, and place. Prior to iEEG implantation, participants completed a behavioral session with both clear and ambiguous (blurred) images. Blurring was titrated to achieve ~50% accuracy, compared to near-ceiling performance for clear images. During intracranial monitoring, participants completed the same task. As expected, ambiguous images led to lower accuracy and longer reaction times. Our previous work using iEEG showed that the ATL increasingly integrates memory signals from the medial temporal lobe (MTL) when perceptual information is ambiguous. In this poster, we extend those findings by focusing on single-unit recordings to examine population-level neural dynamics. Specifically, we analyzed population bursts—brief epochs of coordinated firing—previously shown to encode category information in the ATL (Xie et al., 2024, Nature). We tested whether these bursts resemble evidence accumulation within a decision-making framework. Our results suggest that population bursts represent discrete moments of accumulated evidence, driving decisions regardless of whether inputs are perceptual or memory-based. These findings highlight coordinated population activity as a potential neural mechanism for evidence accumulation in the ATL.

Disclosures: N. Steinberg: None. **M. Riesenhuber:** None. **K.A. Zaghloul:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.05/RR15

Topic: I.10. Human Learning and Cognition

Support: NIH Grant R21MH133055

Title: Temporal features of fronto-limbic circuitry underlying learning-related changes in response to threat

Authors: *M. A. OATES[1], R. CHATFIELD[2], A. FOBIAN[4], R. FONTENOT[4], D. C. KNIGHT[7], J. F. MAGNOTTI[8], A. R. NOLAN[5], J. SZAFLARSKI[3], A. M. GOODMAN[6];
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Abstract: The ability to respond adaptively to threats is an important survival mechanism. A well-established index of emotion regulation is a decreased neurobehavioral response following

learned associations between warning cues and imminent threats. This process may be facilitated by a fronto-limbic network involving the prefrontal cortex (PFC), insula, amygdala, and hippocampus. However, the temporal features of this network remain unclear because prior work has been limited by low temporal resolution of functional MRI (fMRI). Stereo EEG (sEEG) can extend this model by assessing deep brain regions at a high temporal resolution, providing critical timing and target information to guide causal brain mapping of emotion regulation processes. This study aimed to (1) replicate learning-related changes in response to threat using skin conductance response (SCR) and high frequency activity (HFA), and (2) identify the time courses of these neural responses using sliding time windows. SCR and sEEG data were collected while participants (N=15) completed an auditory Pavlovian fear conditioning paradigm. During an acquisition phase (75 trials), threats (100-dB white noise; 0.5s) were presented alone (unpredictable) or co-terminated with a 8s warning cue (predictable). Learning-related reductions in response to threat, or regulation, was defined as decreased responses to predictable versus unpredictable threats. R Analysis and Visualization of EEG (RAVE) was used to analyze sEEG data. HFA was quantified as 40-150 Hz. Electrodes in the amygdala, hippocampus, anterior insula, and PFC were selected for analysis. A 1s analysis window was used for Aim 1. Sliding time windows of 300 ms duration and 50 ms overlap were used for Aim 2. Learning-related reductions in response to threat were observed across the entire task in SCR, and in the dorsolateral PFC (dlPFC), dorsomedial PFC (dmPFC), anterior insula, and hippocampus (all ps < .05). The amygdala showed learning-related reductions in response to threat only during early trials ($p < .05$). Sliding time window analyses revealed that regulation begins earlier (0-550ms) after threat presentation in the dlPFC, dmPFC, and hippocampus, and later (>500ms) in the amygdala (all ps < .05). Prior work has suggested that the PFC and hippocampus act upstream of the amygdala during regulation, thereby functioning as a regulatory mechanism for this network. The present study extends this model by outlining the time courses of these network responses, informing both optimal timing and target stimulation parameters. Modulating these emotion processes holds direct translational potential for treatment of psychiatric disorders.

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Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

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Program #/Poster #: PSTR416.06/RR16

Topic: I.10. Human Learning and Cognition

Support: ZIAMH002032

Title: Chemogenetic inactivation of the entorhinal cortex impairs timing behavior in rhesus monkeys

Authors: *S. WEBSTER-BASS[1], W. WANG[2], M. A. ELDRIDGE[3], W. LERCHNER[4], R. AZADI[1], C. CHANG[5], D. HADJ-MABROUK[6], B. J. RICHMOND[1];
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Abstract: Accurate timing is essential for adaptive behavior, allowing animals to anticipate events, coordinate actions, and optimize decision-making based on temporal expectations. Previous studies in rodents have presented mixed evidence on the role of entorhinal cortex (ERh) in timing behavior, and the causal role of rhesus macaque ERh in timing is currently under-explored. Here, we injected a lentivirus encoding an hM4Di DREADD bilaterally into ERh of one monkey that when activated silences neurons (Lenti-hSyn::hM4Di-CFP, 4 injection sites per hemisphere, 10uL per site at a titer of $2*10^9$ i.u./mL). The monkey initiated a trial in the timing task by holding a touch bar, and waiting for one of four visual cues to appear, each indicating a specific timing interval (0.5 s, 1 s, 2 s, or 4 s). After the cue disappeared, the monkey was required to release the bar after the appropriate time - corresponding to the cued interval - to receive a reward. During pre-virus testing, intramuscular injections of the designer drug deschloroclozapine (DCZ, 0.1mg/kg) had no effects on timing behavior. However, following viral expression, inactivation of neurons within ERh via DCZ led to the monkey frequently releasing the bar early. These results support the possibility that ERh in rhesus macaques is causally involved in timing behavior.

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Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.07/RR17

Topic: I.10. Human Learning and Cognition

Support: OPUS Grant 2019/35/B/HS6/04389

Title: Decoding action timing from single-trial beta rebounds using a transformer-based architecture

Authors: S. BILGIN[1], D. GIOMO[2], ***T. W. KONONOWICZ**[3];
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Abstract: Monitoring errors in temporal behavior is essential for adaptive action, yet the mechanisms by which individuals become aware of such errors remain largely unexplored. While metacognition—the ability to evaluate one's own cognitive processes—has been extensively studied in externally driven perceptual decisions, little is known about how

metacognitive processes operate in the temporal domain.Humans and other animals are able to track time with reasonable accuracy (Kononowicz et al., 2019), albeit with some uncertainty. Evidence suggests that this uncertainty can be integrated into decision-making in a near-optimal manner, implying an ability to monitor and evaluate temporal behavior. Recent findings further demonstrate that individuals can explicitly report their errors in time production along with associated confidence, indicating a degree of awareness of internal timing mechanisms and their informational richness (Bilgin & Kononowicz, 2025; Kononowicz et al., 2019).In this study, we investigated the neural basis of temporal error monitoring on single-trial basis. Participants were asked to produce a 2-second interval by pressing a button twice, then retrospectively evaluate the signed magnitude of their timing error. That is whether they had over- or under-estimated the target interval on a continuous scale. Participants were able to accurately track the signed magnitude of their timing errors, demonstrating a robust self-evaluation.Previous studies have found that beta-band power (15-40 Hz), a neural marker of motor timing, not only predicts the duration of the produced interval but also correlates with the precision of temporal self-evaluation (Kononowicz et al., 2019). In this study, we focus on the dynamics of beta power immediately following the initiation of time production. We predict both the produced duration and its self-evaluation on individual trials using signal denoising techniques based on principal component analysis and transformer architectures. We utilized a Vision Transformer (ViT) to analyze time-frequency representations of EEG signals, treating the data as sequential inputs by segmenting beta rebound activity into overlapping temporal windows (patches). This patch-based approach enables the model to capture long-range dependencies across both temporal and spectral dimensions, facilitating precise decoding of action timing from single-trial dynamics. We also predict the accuracy of self-evaluation using a similar approach. Together, these results demonstrate the role of motor states, indexed by beta oscillations, in predicting motor timing and its self-monitoring.

Disclosures: S. Bilgin: None. **D. Giomo:** None. **T.W. Kononowicz:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

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Program #/Poster #: PSTR416.08/RR18

Topic: I.10. Human Learning and Cognition

Title: Modality distinction and cognitive convergence in a shared low-dimensional subspace during change detection

Authors: H. CHOUBDAR PARVIN[1], K. J. MEADOR[2], ***B. RAZAVI**[3];
[1]McGill Univ., Montréal, QC, Canada; [2]Neurol., [3]Stanford Univ., Palo Alto, CA

Abstract: Change detection, the ability to recognize novel differences in sensory input over time, is essential for adaptive behavior. While auditory and visual change detection tasks engage distinct sensory pathways, they also rely on shared cognitive mechanisms such as attention,

prediction, and working memory. This study examined how these processes are organized across modalities within a shared low-dimensional neural subspace. fMRI component of simultaneous EEG-fMRI data was analyzed from 17 healthy participants performing auditory and visual oddball tasks in separate sessions (OpenNeuro dataset, Walz et al., 2018). Functional connectivity (FC) was computed using Pearson correlations across Glasser atlas regions. Using partial least squares (PLS) regression, we projected the FC matrices into a common low-dimensional subspace with four latent variables (LVs). LV loadings and graph-theoretical metrics were used to interpret network structure. LV loadings revealed two major networks: LV1 included primary sensory, sensorimotor, and spatial attention areas that were present across both modalities but with modality-biased regional centrality; LV2 captured a highly similar network across modalities encompassing default mode, dorsal attention, and frontoparietal systems—key hubs for memory and executive function. These two components were orthogonal, indicating distinct networks facilitating early sensory integration as opposed to higher-order cognitive processing. In contrast, LV3 and LV4 revealed modality-specific enrichment in network connectivity, with enhanced segregation in auditory or visual domains. These findings demonstrate that change detection across modalities engages a combination of shared and modality-biased networks, and that such architectures can be effectively parsed within a low-dimensional connectivity space.

Disclosures: H. Choubdar Parvin: None. **K.J. Meador:** None. **B. Razavi:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.09/SS1

Topic: I.10. Human Learning and Cognition

Support: NSF Grant 2317706

Title: Sleep-dependent memory consolidation in a pilot nap study contributes to the detection of hidden temporal regularities within a community-based pattern

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[1]Univ. of Texas San Antonio, San Antonio, TX; [2]Psychology, The Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Sleep is well-known to improve memory, but it remains unclear how the consolidation of complex temporal information occurs across slow wave sleep (SWS) and rapid eye movement (REM) sleep cycles. Evidence from the serial reaction time task (SRTT) shows that hidden patterns with simple temporal rules are implicitly learned during wake and can transform into explicit awareness after sleep rich with SWS. Here, we present a pilot human study employing a novel SRTT task with a community-structured sequence that offers the potential to investigate the effects of sleep on learning of patterns spanning multiple timescales. In this task, a successive

series of stimuli appeared in one of 7 locations following a hidden temporal pattern where two unique communities, each composed of 3 different locations, were repeatedly visited. While each community was chosen at random, the direction of location visits within a community was determined by previous community visits, thus, requiring participants to utilize recent information across short & long timescales to reach optimal performance. Participants were first trained on SRTT and then completed a generation task where explicit knowledge of the hidden pattern was tested. Following a retention period of either an EEG-monitored 90-minute nap or a 10-minute wake period, they were tested on the generation task again with novel sequences added following the same temporal rules. Results for the generation task prior to the retention period showed that participants were sometimes able to predict locations at the end of a community reflecting partial detection of short temporal regularities, but they did not score above chance when predicting locations in the middle of a community (which depended on the series of recent community visits), suggesting longer temporal regularities were not detected after initial learning. Following the retention period, the nap group, but not the 10-minute group, showed improved accuracies predicting short temporal regularities in familiar sequences that also transferred to the novel sequences. However, no improvement was evident for either group for the longer temporal regularities. These results suggest that explicit learning of the community-structured SRTT pattern is enhanced already after a few SWS-REM cycles but is limited to regularities with shorter timescales. In future studies, we predict that longer sleep periods with multiple SWS-REM cycles may extend performance improvement to show learning of longer temporal regularities.

Disclosures: M. Gonzales: None. **I. Lerner:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.10/SS2

Topic: I.10. Human Learning and Cognition

Title: The impact of rhythm training on interpreting Shakespearean literature

Authors: *M. I. BURRITT ST. ANGELO;
Neurosurger, Loyola Univ. Med. Ctr., Chicago, IL

Abstract: Throughout their academic careers, students face the intimidating task of interpreting the works of William Shakespeare. Understanding his iconic themes profoundly enriches student comprehension of the humanities. However, many students struggle to connect with Shakespeare's texts due to their unfamiliar style. Previous literature has shown that recognizing rhythmic patterns improves reading comprehension. In this study, we determine if familiarity with rhythmic cues impacts students' perception of Shakespearean language. Participants were students ages 18-24 enrolled in a post-secondary educational program. We collected information regarding participants' prior rhythmic practice including, but not limited to, extracurricular

engagement in music, dance, or poetry. In their first session, participants were instructed to mimic a series of rhythmic patterns and were scored for accuracy. Over the course of two consecutive sessions, participants read a collection of "Shakespearean," "other Old English," and "common vernacular" texts. The included texts were selected by literary experts unaware of the experiment hypothesis. Additional information regarding the structure and composition of the texts including text length, rhyme scheme, and low-frequency word use, was recorded. After reading each text, participants responded to prompts which were then scored by a predetermined rubric to gauge participant comprehension of the texts. A preliminary, correlational analysis found that participants with prior experience in rhythmic activities were generally superior at mimicking and maintaining patterns during the rhythmic tests, an expected result. However, our results further unveil a correlation between participants' ability to mimic rhythm and their literary analysis scores. Students superior with rhythm were also superior in identifying core aspects of Shakespearean texts, such as theme and tone. These findings emphasize the impact of rhythmic patterns on literary analysis and suggest that students' experiences with rhythm influence their interpretations of Shakespearean works. The future of this study considers the neural connectivity of rhythm and reading by comparing fMRI measured neural activity between baseline, "rhythmic" text, and "non-rhythmic" text readings. Future studies will ask if inclusion of rhythm training in academic contexts influences the interdisciplinary benefits students receive from rhythm training, and look to understand how differences in sensation and perception, particularly across intellectual and development disabilities, affects the impact of rhythm training on reading comprehension.

Disclosures: M.I. Burritt St. Angelo: None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.11/SS3

Topic: I.10. Human Learning and Cognition

Title: Resting-state EEG oscillations and their association with prospective memory performance

Authors: *T. S. WARREN, E. BABY, S. ROGERS, R. A. MUDAR;
Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: A growing body of research suggests that resting state electroencephalography (EEG) oscillations at alpha and beta frequencies are linked to cognitive performance on tasks of verbal recognition, attention, and working memory. However, little is known about the association between resting state EEG oscillations and prospective memory (PM), which is the ability to plan and execute intended action(s) in the future. To investigate this relationship, we explored the association between resting state absolute power in theta, alpha, and beta frequencies and PM behavioral performance in neurotypical young adults. A total of 18 neurotypical young adults (14

F/4 M; age = 20.39 ± 1.97 years; education = 14.11 ± 1.91 years) underwent EEG recording during an eyes-closed resting state condition. Absolute power at theta (4-8 Hz), alpha (8-12 Hz), and beta (13-30 Hz) were calculated via Fourier transformation-based spectral analysis at the following midline electrode: Fz, FCz, Cz, CPz, and Pz. Participants completed two novel cue-based PM tasks: Animal cued-Prospective Retrieval Task (Ac-PRT) and Object cued-Prospective Retrieval Task (Oc-PRT). During the tasks, participants were instructed to form PM intentions (Cue trials), retain PM intentions during ongoing tasks involving semantic judgement (Ongoing trials) while inhibiting responses to distractors (Lure trials), and retrieve PM intentions based on previously encoded intention (PM Retrieval trials). Reaction times and accuracy were recorded across trial types. Preliminary analyses showed that higher accuracy in PM Retrieval trials on the Oc-PRT correlated with higher resting state absolute power at alpha in FCz, $r(16) = 0.56$, $p = .016$, Cz, $r(16) = 0.59$, $p = .011$, CPz, $r(16) = 0.58$, $p = .012$, and Pz, $r(16) = 0.55$, $p = .018$, and beta in Pz, $r(16) = 0.54$, $p = .021$. Previous studies have observed positive associations between alpha oscillations and verbal recognition, and beta oscillations with attention and working memory in young adults. Interestingly, the associations between resting state EEG oscillations and PM behavioral performance were only seen in Oc-PRT and not in Ac-PRT. Given the underlying differences in feature processing and neural representations of animals versus objects, it is plausible that the relationship between resting state EEG and PM may differ based on the semantic categories involved in the tasks. These preliminary findings serve as a benchmark for exploring the interrelationship between resting state EEG and PM tasks involving semantic processing.

Disclosures: T.S. Warren: None. **E. Baby:** None. **S. Rogers:** None. **R.A. Mudar:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

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Program #/Poster #: PSTR416.12/SS4

Topic: I.02. Perception and Imagery

Title: The role of right supplementary motor area and posterior parietal cortex in beat-based temporal prediction: evidence from a TMS-EEG study

Authors: *Z. CHENG[1], R. BALASUBRAMANIAM[2];
[1]Univ. of California, Merced, Merced, CA; [2]Cognitive & Information Sci., Univ. of California, Merced, Livermore, CA

Abstract: Beat perception is a core component of temporal cognition that enables humans to extract a regular pulse from rhythmic auditory input and anticipate the timing of future events. While this process believed to be an auditory perceptual phenomenon, recent neuroimaging studies suggest the involvement of sensorimotor networks, even in the absence of overt movement.The current study investigated the role of the right SMA and right PPC in beat perception using transcranial magnetic stimulation (TMS) and electroencephalography (EEG).

While the PPC is proposed to play a role in aligning internal motor-based predictions with external rhythmic input, the SMA was not explicitly included in the model but was commonly found activated in beat perception studies.We applied continuous theta burst stimulation to the right PPC and right SMA in separate sessions, along with a sham condition, to test their respective roles in a beat alignment task. Participants completed two subtests of the Adaptive Beat Alignment Test (A-BAT): one probing tempo-based timing sensitivity (inter-beat interval, IBI) and the other assessing phase-based timing alignment. EEG data were collected before and after TMS while participants passively listened to musical stimuli and made predictions of upcoming beat patterns. We analyzed event-related spectral perturbations (ERSP) in alpha and beta bands over right motor-parietal electrodes (FC2, CP2), time-locked to every beat onset.Behavioral results revealed no significant changes in performance following either right SMA or PPC stimulation, though phase detection thresholds increased numerically after PPC stimulation. ERSP analyses revealed significant modulation of oscillatory dynamics following PPC stimulation. Specifically, alpha power increased significantly after PPC stimulation ($F(1,3) = 9.214$, $p = .039$), and beta power decreased ($F(1,3) = 10.635$, $p = .032$), indicating a disruption in the sensorimotor prediction network. No significant changes in alpha or beta power were observed in the SMA or sham conditions.These early results support the idea that the right PPC may play a critical role in sensorimotor alignment and predictive timing, consistent with its proposed function within the dorsal stream. The absence of significant SMA effects may reflect its context-dependent role, potentially engaged more strongly in conditions requiring endogenous beat maintenance or increased prediction demands. This ongoing study contributes to ongoing efforts to refine our understanding of the neural mechanisms underlying temporal prediction.

Disclosures: Z. Cheng: None. **R. Balasubramaniam:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.13/SS5

Topic: I.10. Human Learning and Cognition

Support: JSPS KAKENHI (25H01096)

Title: Selective assignment of a supplementary motor response assists Bayesian estimation with two independent priors for timing behavior in virtual baseball batting

Authors: *T. IWANAGA[1], Y. TANAKA[1], J. SUZUKI[1], M. MIYAZAKI[1,2], T. KIMURA[3]; [1]Grad. Sch. of Integrated Sci. and Technology, Shizuoka Univ., Hamamatsu city, Japan; [2]Facility of Informatics, Shizuoka university, Hamamatsu city, Japan; [3]NTT Communication Sci. Labs., Atsugi-Shi, KANAGAWA, Japan

Abstract: According to the Bayesian estimation model, the accuracy of sensorimotor behavior can be improved by integrating prior distribution of the target with sensory signals.

Psychophysical studies have demonstrated that human timing behavior is consistent with this model. In daily tasks, we encounter multiple events (e.g., fastball/slowball in ball sports), each with unique statistics. As such, the brain should acquire multiple prior distributions in the real world. We recently found that supplementary motor responses (SMR) facilitate the acquisition of two independent prior distributions (short and long intervals) in timing tasks. Participants effectively acquired two independent prior distributions by selectively adding a non-dominant hand or a vocal response concomitantly with the dominant-hand timing response for one of the priors (Natsume et al. 2025, Proc R Soc B). Moreover, this facilitation of multiple prior acquisitions was increased by adding a supplementary vocal response immediately before the dominant-hand timing response (Okumura et al. Neuroscience 2024). In this study, we tested whether SMR can assist multiple-prior acquisition of timing behavior in a virtual baseball batting task. Each participant performed 240 trials of the virtual batting task in which they could hit balls if their swing were correctly timed with the ball's arrival. The time interval from the pitcher releasing the ball to the ball reaching the home base (target time interval) was sampled from either the short prior (500-1000 ms, fastball) or long prior distribution (1125-1625 ms, slowball). Before starting each trial, the stadium bulletin board behind the pitcher was colored blue or red, indicating whether the target time interval was sampled from a short or long prior. The participants were divided into the SMR and non-SMR groups. The SMR group selectively switched the buttons on the virtual bat controller immediately before swinging the virtual bat for one color (i.e., prior). The non-SMR group swung the virtual bat without switching buttons in all trials, irrespective of the color. Consequently, the divergence between the two acquired priors was greater in the SMR group than in the non-SMR group. Our findings suggest that SMRs also assist the acquisition of multiple prior distributions of daily timing behaviors with greater degrees of freedom than conventional psychophysical tasks.

Disclosures: T. Iwanaga: None. **Y. Tanaka:** None. **J. Suzuki:** None. **M. Miyazaki:** None. **T. Kimura:** None.

Poster

PSTR416: Temporal Dynamics and Internal States in Memory, Prediction, and Action

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR416.14/SS6

Topic: I.10. Human Learning and Cognition

Support: NINDS U01 NS121472
RS-2023-00211018
IBS-R015-D1

Title: Mental Data Structuring in Human Hippocampus During One-shot Sequence Learning

Authors: *S. SON[1], H. AZAB[2], M. EL-GABY[7], M. SABLÉ-MEYER[8], S. SHAH[3], R. MATHURA[4], E. BARTOLI[5], A. WATROUS[6], A. ANAND[6], J. ADKINSON[6], T. DONOGHUE[9], U. TOPALOVIC[11], J. J. SAKON[12], Z. KURTH-NELSON[14], E. H. SMITH[15], N.

A. SUTHANA[13], I. FRIED[16], J. JACOBS[10], M. M. BOTVINICK[17], T. E. BEHRENS[18], S. M. YOO[19];
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Abstract: Humans can learn event sequences from just one experience. Previous studies explained sequence encoding as the association of events with sequence labels (e.g., time cells). However, it remains unclear how the brain, particularly at the populational level, flexibly re-associates sequences from extremely limited experience. Here, we investigated the neural population mechanisms underlying one-shot sequence learning. During the experiment, participants selected four target locations in a specific order on a grid. Without knowing the correct order, participants initially learned through trial and error in the first trial, but subsequently executed the correct sequence almost perfectly, demonstrating one-trial learning. Simultaneously, we recorded single-unit activity through stereoelectroencephalography (sEEG) across multiple brain regions. We found that during learning, a large portion of hippocampal neurons increased their firing rate linearly with the progression of the sequence. These neurons explained the most variance in population activity, prioritizing sequence encoding along a single linear axis over other sources of variability, thereby making it more resistant to noise (i.e., line-like attractor). Interestingly, the second major group of neurons fired only during the correct progression of the sequence, regardless of the specific sequence events. These neurons exhibited activity nearly orthogonal to the linear sequence axis, perturbing the population activity to trigger the transition from one event to the next. This interaction between the linear sequence axis and trigger neurons was absent in other brain regions, including the entorhinal cortex, anterior cingulate cortex, and orbitofrontal cortex. After one-shot learning (i.e., from the second trial onward), the activity of these two major cell groups declined rapidly. After learning, the firing rates at each event's end resembled those at the start of the next, including across the transition from the end of the previous trial's sequence to the beginning of the current one, forming a loop structure in population space (i.e., ring-like attractor) This illustrates the brain's flexibility in shifting from linear encoding to nonlinear activity based on learning demands, challenging the conventional view that neural dynamics are invariant within a single task. Our study also highlights how data structuring methods, such as queues used in computers, parallel the neural mechanisms involved in sequential processing during one-shot learning.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.01/SS7

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH RM1MH138309
T32NS115667
TSF2019F

Title: Mapping spatial molecular signatures of brain aging with an enhanced spatial transcriptomics analysis pipeline

Authors: *D. PARMAKSIZ[1], S. MANJILA[2], D. SHIN[3], I. E. BJERKE[4], A. PAUL[5], Y. KIM[6]; [1]Penn State Col. of Med., Hershey, PA; [2]neural and behavioral Sci., NBS, Penn State Col. of Med., hershey, PA; [3]Neural and Behavioral Sci., Penn State Col. of Med. Dept. of Neural and Behavioral Sci., Hershey, PA; [4]Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway; [5]Cold Spring Harbor Lab. Press, Cold Spg Hbr, NY; [6]Neural and Behavioral Sci., Penn State Univ., Hershey, PA

Abstract: Aging alters the brain at molecular and cellular levels, contributing to cognitive decline and increased vulnerability to neurodegeneration. Spatial transcriptomics enables the study of these changes in situ, but most current analysis workflows—largely adapted from single-cell RNA-seq—fail to account for the biological and technical complexities unique to spatial data. Here, we developed a modular and enhanced analysis pipeline tailored to multiplexed error-robust fluorescence in situ hybridization (MERFISH), using a mouse brain aging dataset as a test case. We analyzed 12 coronal brain sections from young (2 months) and aged (22 months) male C57BL/6J mice, spanning anterior to posterior regions and processed with a custom 500-gene MERFISH panel on the Vizgen MERSCOPE platform. The pipeline integrates improved segmentation, registration to the Allen Common Coordinate Framework, and quality filtering, followed by two analysis branches: one that performs hierarchical clustering to identify and refine cell type and subtype annotations, and another that models differential gene expression (DGE) across age groups. To address scale uncertainty in spatial transcriptomics, where total transcript counts vary widely across cells, we adapted the ALDEx2 framework to estimate gene expression changes using a Dirichlet-multinomial model with Monte Carlo sampling. Unlike methods that normalize or regress out total counts and volume, our compositional approach preserves relative abundance patterns without assuming equal scale. For aging, we incorporated a biologically informed adjustment for ~20% RNA decline in older cells, based on reduced RNA Polymerase II activity. This correction improved both the interpretability and biological relevance of DGE results. Our approach reduces false positives, preserves meaningful variation, and improves interpretation of aging-related expression changes. Spatial mapping of DGE results reveals region- and cell-type-specific patterns of vulnerability. For example, in microglia, we identified significant age-related decreases in genes that suppress neuroinflammation and limit microglial activation. These findings illustrate the power of our

pipeline to detect subtle, biologically relevant changes. By addressing key challenges in spatial transcriptomics, our method improves the rigor and resolution of brain aging studies.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.02/SS8

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: UM1MH130994

Title: Chromatin Dynamics and DNA Methylation in Single Cells from Human Basal Ganglia

Authors: *W. DING[1], C. T. BAEZ BECERRA[2], Y. FU[3], M. BEHRENS[4], J. R. ECKER[5]; [1]Genomic Analysis Lab., [2]Computat. Neurobio. Dep, Salk Inst. for Biol. Studies, San Diego, CA; [3]Allen Inst., Seattle, WA; [4]CNL-B, The Salk Inst. for Biol. Studies, La Jolla, CA; [5]PBIO-E, Salk Inst., La Jolla, CA

Abstract: The basal ganglia are interconnected brain structures essential for voluntary motor control and reward processing, and their dysfunction contributes to neurological and psychiatric disorders. We present the first multimodal single-cell epigenomic atlas of the human basal ganglia, integrating DNA methylation, 3D chromatin conformation, and spatial transcriptomics across cell types and multiple basal ganglia subregions. This resource reveals striking regional epigenetic variation within shared cell types and uncovers principles of chromatin organization that differentiate neuronal and non-neuronal cells, providing a framework for understanding the molecular basis of brain disorders rooted in epigenomic dysregulation. Here, we profiled 203,036 single nuclei from eight dissected basal ganglia subregions using snm3C-seq to jointly measure DNA methylation and 3D chromatin conformation. We integrated these data with prior snm3C-seq and snmC-seq datasets to assemble a joint epigenomic map comprising 267,662 cells, 1,247 clusters, and 30 cross-modality-defined subclasses from seven donors. We identified millions of differentially methylated regions (DMRs) that were cell-type- and region-specific, enriched for distinct transcription factor motifs. These DMRs spanned genes and regulatory elements, revealing epigenetic heterogeneity not only across cell types but also among spatially distinct regions within the same cell type. Spatial transcriptomics using a 920-gene MERFISH panel validated anatomical dissection, uncovered spatial epigenetic gradients, and linked methylation diversity to transcriptional output. Chromatin conformation analysis revealed cell-type- and region-specific interaction patterns. Neurons exhibited longer-range contacts, stronger A/B compartmentalization, expanded topologically associating domains (TADs), and reduced domain strength relative to non-neuronal cells. Notably, the globus pallidus displayed a lower BB/AA ratio, larger TADs, and weaker insulation—hallmarks of a more euchromatic genome

organization. By integrating chromatin accessibility and histone modification data, we constructed cell-type-resolved enhancer- and repressor-promoter networks, enabling a systematic view of gene regulation in 3D genome architecture. Finally, we delineated associations between methylation, chromatin interaction, and chromatin accessibility across regulatory contexts. This study provides a comprehensive resource for exploring the spatial and regulatory genome organization of the human brain's basal ganglia, offering new insights into the epigenetic basis of brain disease.

Disclosures: W. Ding: None. **C.T. Baez becerra:** None. **Y. Fu:** None. **M. Behrens:** None. **J.R. Ecker:** None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.03/SS9

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Advancing Neural Tissue Segmentation for Spatial Transcriptomics in the Human Brain

Authors: *L. WU, A. WARDHANI, J. T. PHAN, A. HECK, K. YOUNG, Y. CUI, S.-L. ROGERS, K. TON, J. BEECHEM;
Bruker Spatial Biol., Seattle, WA

Abstract: Spatial transcriptomics is rapidly transforming neuroscience by enabling the in situ mapping of gene expression with cellular resolution. This approach offers unprecedented opportunities to study the molecular architecture of neural circuits, identify cell-type-specific transcriptional programs, and uncover spatially localized mechanisms underlying development, function, and disease. However, its full potential hinges on accurate cell segmentation—particularly challenging in brain tissue, where cells exhibit complex, overlapping morphologies. To overcome these challenges, we developed a specialized segmentation pipeline tailored for the cellular intricacies of brain tissue. Central to this pipeline is a machine learning model trained primarily on neural tissue. By incorporating nuclear, neuronal, and glial staining across three morphological channels, the model captures critical distinctions between intermingled cell types. Training datasets were curated to represent a balanced spectrum of neural cell classes and environments, minimizing morphological bias. Standard segmentation methods often mislabel or omit extended neuronal processes and cells of unusual size and shape such as Purkinje neurons. To address this, we designed a neuroscience-aware post-processing workflow that includes auto-thresholding and spatial connectivity analyses to recover missed structures, followed by distance-based pixel assignment to ensure accurate inclusion of distal processes to their cell origin. When applied to CosMx® spatial transcriptomics data from FFPE human brain tissue, the pipeline yielded a 17% increase in transcripts per cell with minimal changes to cell volume and a 23% reduction in over-segmentation, without inflating extracellular transcript noise. These improvements reflect more accurate delineation of neural cell boundaries and suggest a more

faithful representation of cell-specific transcriptomes. By tailoring segmentation tools to the unique demands of brain tissue, this work supports more accurate spatial transcriptomic analysis in brain tissue and may help enable finer-grained molecular investigations of neural structure and function.

Disclosures: L. Wu: A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **A. Wardhani:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology. **J.T. Phan:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **A. Heck:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **K. Young:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **Y. Cui:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **S. Rogers:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **K. Ton:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **J. Beechem:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bruker Spatial Biology.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.04/SS10

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatially Resolved Multiomics Profiling of Glioblastoma Reveals Immune-Tumor Microenvironment Architecture

Authors: *Y. CUI[1], C. WILLIAMS[1], C. LEE[1], C. PHAN[1], S. MCKINZIE[1], S. HE[1], A. HECK[2], H. ZHAI[1], J. LYSSAND[3], P. DIVAKAR[4], **J. BEECHEM**[1];
[1]Bruker Spatial Biol., Seattle, WA; [2]Bruker Spatial Biol., Seattle, WA, ; [3]Seattle, WA, ; [4]Lewisville, TX.

Abstract: Glioblastoma (GBM) is a highly heterogeneous and aggressive brain tumor with dismal prognosis, necessitating advanced approaches to decode its complex biology. Here, using the CosMx® Spatial Molecular Imager, we performed high-resolution same-slide spatial multiomics profiling on tissue sections from a GBM patient. The dataset includes expression data for over 18,000 transcripts (covering over 99% of protein-coding genes) and ~70 proteins associated with tissue morphology and immune-oncology pathways, allowing for an integrated analysis of gene and protein expression across tumor and peri-tumoral regions. With transcriptome-wide RNA information, we identified 5 distinct GBM tumor cell subtypes - astrocyte-like, OPC-like, mesenchymal-like, stem cell-like and actively proliferating cells - each

exhibiting unique gene expression signatures and spatial distributions, providing an opportunity to investigate spatial gradients of gene and protein expression relative to histopathological features. Concurrent transcriptomic and proteomic profiling, including key markers (CD3, CD4, CD45, CD68, vimentin, fibronectin, pan-RAS, EGFR, among others), revealed spatially restricted immune, stromal and tumor-specific expression patterns, elucidating tumor-microenvironment interactions. Differential expression analyses suggest distinct molecular signatures between the tumor core and the invasive margin. Leveraging a data-driven approach, we integrated these multiomics datasets to uncover spatially defined molecular networks driving tumor progression, invasion, and immune evasion. Notably, spatial mapping of drug resistance markers (e.g., EGFR, B7-H3) and immune checkpoints (e.g., PD-L1, IDO1) highlighted therapeutic vulnerabilities within distinct tumor niches. This single-sample dataset demonstrates the potential of high-plex spatial multiomics to dissect the tumor-immune microenvironment in GBM at unprecedented resolution. While limited to one case, this work highlights a scalable framework for spatially resolved molecular analysis of brain tumors. By integrating transcriptomic and proteomic data with spatial context, this approach offers a powerful lens for uncovering mechanisms of immune evasion, tumor heterogeneity, and microenvironmental organization - laying the groundwork for future multi-sample studies aimed at therapeutic stratification and biomarker discovery in glioblastoma.

Disclosures: Y. Cui: A. Employment/Salary (full or part-time): Bruker Spatial Biology. **C. Williams:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **C. Lee:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **C. Phan:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **S. McKinzie:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **S. He:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **A. Heck:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **H. Zhai:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **J. Lyssand:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **P. Divakar:** A. Employment/Salary (full or part-time): Bruker Spatial Biology. **J. Beechem:** A. Employment/Salary (full or part-time): Bruker Spatial Biology.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.05/SS11

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: the National Key R&D Program of China: 2022YFA1603604
the National Key R&D Program of China: 2022YEF0203200

Title: Cross-species comparison of single-cell spatial transcriptomic atlases of the thalamus

Authors: L. HAN[1], N. YUAN[2], T. LUO[3], M. WANG[4], Q. TAO[5], Z. LAI[1], J. MAORUI[1], M.-M. POO[6], ***Z. SHEN**[6];

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Abstract: The evolutionary changes in cellualr trancriptomic profile and composition of the primate thalamus remains poorly understood. Single-nucleus RNA sequencing of over 0.5 millioin macaque thalamic cells identified more than 60 transcriptome-defined cell types. Comparing among human, macaque, and mouse transcriptomic data, we identified the evolutionary conservation and divergence of the thalamic nuclei and revealed primate-specific glutamatergic and GABAergic cell types. Single-cell spatial transcriptomes delineated the precise transcriptome-defined parcellation boundaries between thalamic nuclei and uncovered systematic patterns in cell type distribution across thalamic subdivisions. Several primate-specific glutamatergic cell types were found in the higher-order thalamic nuclei and connected to specific brain regions. Integration with GWAS data revealed several thalamic nuclei with neuropsychiatric disorders. These comprehensive multi-species cellular and spatial atlases of thalamus advanced our understanding of its evolution and function.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.06/SS12

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Massively parallel single cell transcriptomics reveals drug responses in a 4 million cell neuro-oncology cohort within a 100 million cell atlas

Authors: *J. PANGALLO, V. TRAN, S. MARRUJO, S. SCHROEDER, O. KAPLAN, G. DEMIRKAN, C. ROCO, A. ROSENBERG, K. FEDDER-SEMMES;
Parse Biosci., Seattle, WA

Abstract: Understanding the acute transcriptional consequences of drug exposure is essential for elucidating drug mechanisms of action and accelerating the development of therapies in the neuro-oncology drug discovery space. Massively scalable single cell RNA-seq now makes it possible to capture those acute responses across thousands of drug-cell interactions in a single experiment, transforming what was once a gene by gene screen into a population scale view of drug activity. To showcase this ability, we leveraged combinationarial barcoding to build a 100 million cell single cell RNA-seq perturbation atlas with Tahoe Therapeutics' Mosaic platform. Within it, a focused four million cell cohort represents four clinically relevant neural cancer

models—glioblastoma A-172, neuroglioma H4, astrocytoma SW 1088, and neuroblastoma CHP-212. These models, along with 46 other additional human cell lines, were treated with 379 drug compounds at three concentrations for twenty-four hours, chemically fixed, and iteratively barcoded in batches of roughly 10 million cells on the Parse Biosciences GigaLab platform. Automated library construction across 96-well plates produced sequencing-ready material for all 100 million plus cells in under five weeks. Sequencing on the UG 100™(Ultima Genomics) system followed by Demuxlet demultiplexing yielded high-resolution transcriptomes for every cell and unambiguously assigned each cell to its original line. The neural cancer subset alone spans 4,540 cell-line-drug-dose conditions, capturing the diverse transcriptional programs and drug-response landscapes of glioma, astrocytoma, neuroglioma, and neuroblastoma models at single cell resolution. Processing fixed cells in large pools minimized batch effects, enabling direct, large-scale comparisons of drug perturbations across the entire dataset.This study demonstrates that single cell combinatorial barcoding can transform modest scRNA-seq experiments into atlas level datasets, delivering the statistical power required to model complex therapeutic responses in neuro-oncology and beyond. The resulting 4 million cell neural cancer dataset nested within a 100 million cell atlas establishes a new benchmark for high-throughput drug perturbation modeling and provides a versatile resource for future mechanistic and translational research.

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Poster

PSTR417: Spatial and Multi-omics II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.07/TT1

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Enhanced Spatial Transcriptomics Enables High-Resolution Profiling of Neuronal Transcription Factors in the Developing Nervous System

Authors: *R. CHEN, M. RAY, J. HE, B. WANG, B. YANG, T. WIGGIN, L. MAZIASHVILI, B. AUCLAIR, J. HE, G. EMANUEL;
Vizgen, Cambridge, MA

Abstract: The advent of imaging-based high-plex spatial transcriptomics tools with single-cell resolution has transformed our understanding of the structure, function, and development of the nervous system. These technologies provide the ability to visualize gene expression in its native spatial context, enabling deeper insights into cellular identity, organization, and communication. However, detecting genes expressed at low levels—especially in samples with compromised RNA quality—remains a challenge for downstream biological analysis. Transcription factors, in particular, are difficult to profile due to their typically low abundance, transient expression, and

tight regulation across developmental stages. To address this, the Multiplexed Error-Robust Fluorescence In Situ Hybridization 2.0 (MERFISH 2.0) chemistry and sample preparation workflow were developed to enhance transcript detection efficiency for up to 1,000 genes. This technology enables highly sensitive, spatially resolved RNA profiling directly in tissue, while preserving cellular architecture and gene expression patterns. In this study, we applied MERFISH 2.0 to comprehensively profile transcription factors during mouse development. Two panels, each containing 815 genes, were designed to cover the full repertoire of known mouse transcription factors. Multiple developmental stages of mouse embryos and postnatal brains were sectioned onto MERSCOPE Ultra® slides and analyzed using both panels. The resulting data enabled detailed spatial and single-cell analyses, demonstrating that MERFISH 2.0 provides robust detection of transcription factors throughout embryonic and early brain development. High-resolution, spatially resolved profiling of transcription factors at the single-cell level opens new avenues for studying neuronal development, function, and disease. This approach will facilitate exploration of transcriptional dynamics that drive lineage commitment, neurogenesis, and brain regionalization, offering a powerful tool for investigating gene regulation in complex tissues.

Disclosures: R. Chen: A. Employment/Salary (full or part-time): Vizgen. **M. Ray:** A. Employment/Salary (full or part-time): Vizgen. **J. He:** A. Employment/Salary (full or part-time): Vizgen. **B. Wang:** A. Employment/Salary (full or part-time): Vizgen. **B. Yang:** A. Employment/Salary (full or part-time): Vizgen. **T. Wiggin:** A. Employment/Salary (full or part-time): Vizgen. **L. Maziashvili:** A. Employment/Salary (full or part-time): Vizgen. **B. Auclair:** A. Employment/Salary (full or part-time): Vizgen. **J. He:** A. Employment/Salary (full or part-time): Vizgen. **G. Emanuel:** A. Employment/Salary (full or part-time): Vizgen.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.08/TT2

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: The Multipro® Human Discovery Panel Unlocks Comprehensive Multiomics Single-Cell Profiling

Authors: *I. GODINEZ;
Proteintech Genomics, SAN DIEGO, CA

Abstract: Advancing the understanding of neurobiology requires tools that capture the cellular and molecular diversity of the human nervous system. The Multipro® Human Discovery DNA-barcoded Antibody Panel seamlessly integrates with the 10x Genomics Chromium Single-cell Gene Expression Flex workflow, providing a comprehensive and highly sensitive approach for profiling expression of over 326 validated protein biomarkers at single-cell resolution. With an expansive selection of antibodies against cell surface and intracellular proteins, this pre-titrated

panel enables researchers to dissect the cellular and molecular mechanisms underlying neuroimmune responses, autoimmunity, and immunotherapies. Here we present data to demonstrate the utility of high-plex DNA-barcoded antibody panels in neurobiology discovery work by exploring relevant protein and gene expression networks.

Disclosures: I. Godinez: None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.09/TT3

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: The accuracy of cell type labels transferred to a MERFISH atlas of the adult mouse brain

Authors: *J. WATERS;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Cell type taxonomies have advanced our understanding of how cells are organized into and contribute to the functions of biological tissues and organs. Many cell type taxonomies are based on gene expression measured with high throughput single cell RNA sequencing (RNAseq), resulting in taxonomies that, for heterogeneous tissues such as mammalian brain, may include thousands of cell types described by the expression of tens of thousands of genes. A new study may include millions of cells, each of which needs to be matched to a cell type from the taxonomy, permitting transfer of a cell type label from taxonomy to cell. Many strategies and software tools for label transfer are available. Benchmarking studies have compared the labels transferred by these tools and the similarity of gene expression between cell and cell type, but rarely the proportion of transferred labels that are accurate. I have estimated the accuracy of cell type labels transferred from an RNAseq taxonomy to a publicly available atlas of the adult mouse brain generated with the multiplexed error-robust fluorescence in situ hybridization (MERFISH) cellular spatial transcriptomics method (published in Yao *et al.* (2023) Nature 624, 317-332 and available through the Allen Brain Cell Atlas and via Github, https://alleninstitute.github.io/abc_atlas_access/intro.html). I generated pseudo-ground truth labels by simulating a MERFISH dataset from RNAseq results and calculated the probability that each label is accurate. Further analyses revealed that inaccurate labels result from deficiencies in the label transfer routine rather than systematic differences in expression measured with RNAseq and MERFISH, that the accuracy of label transfer varies substantially across cell types, and that small numbers of distractor genes can have an outsized impact on the accuracy of label transfer. I conclude that with adequate care, mostly accurate identification of even fine-grained cell types is possible with modern spatial transcriptomics technologies.

Disclosures: J. Waters: None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.10/TT4

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: RS-2024-00406240

Title: Ultra-multiplexed imaging for spatial proteomics analysis of the brain

Authors: *J.-B. CHANG;
Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Understanding the spatial organization of proteins in the brain is critical for unraveling its complex biology. To address the growing need for high-resolution spatial proteomics, a range of advanced multiplexed imaging technologies has been developed and increasingly applied in neuroscience research. In this study, we employ several state-of-the-art ultra-multiplexed imaging methods to map the spatial distribution of a wide array of proteins across the brain, enabling comprehensive insights into its molecular architecture.

Disclosures: J. Chang: A. Employment/Salary (full or part-time): Flashomics Corp..

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.11/TT5

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Simultaneous single cell sequencing of genetic and epigenetic bases

Authors: *P. CREED;
biomodal, Cambridge, United Kingdom

Abstract: A growing body of evidence highlights the critical role of epigenetic modifications in shaping neural identity and function. In the brain, where cellular diversity and plasticity are paramount, DNA modifications such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) serve as key regulators of gene expression. These cytosine modifications influence neuronal development, synaptic plasticity, and are implicated in a range of neurological disorders. While 5mC is traditionally associated with gene repression, 5hmC—particularly enriched in neuronal tissues—is increasingly recognized as a marker of active gene regulation and enhancer activity.

Despite their importance, resolving the distribution and interplay of 5mC and 5hmC at single-cell resolution in the brain has remained a major technical challenge. We present a single nuclei workflow to simultaneously determine 5mC and 5hmC at the single molecule level, based on an existing bulk assay for simultaneous measurement of genetics, 5mC, and 5hmC.
Applying this method to mouse cortical tissue, we identified multiple neural cell populations based on their unique 5mC and 5hmC landscapes. Integrating this data with existing single-cell ATAC-seq and RNA-seq atlases, we were able to see distinctive patterns of 5mC and 5hmC marking expressed genes. These findings underscore the power of single-cell epigenomic profiling to uncover hidden regulatory diversity in the brain.

Disclosures: P. Creed: None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.12/TT6

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF DBI 2119968

Title: Transcriptome profiling of roseolovirus-infected differentiated neuroblastomacells: A comparison between HHV-6A versus HHV-6B infected SH-SY5Y cells.

Authors: *A. BAJPAI[1], E. BAHRAMIAN[2], R. CEBALLOS[3];
[1]Quantitative and Systems Biol., Univ. of California, Merced, MERCED, CA; [2]Mol. and Cell Biol., Univ. of California, Merced, Merced, CA; [3]Mol. and Cell Biol., Univ. of California Merced, Merced, CA

Abstract: The roseoloviruses human herpesvirus-6A and -6B (HHV6-A and HHV-6B) have been implicated as etiologic agents for a variety of neurological disorders including: epilepsy, multiple sclerosis, and chronic fatigue syndrome. However, the mechanisms and molecular basis by which roseolovirus infections induce neural dysfunction remains poorly understood. In this study, we examine the transcriptome profiles (e.g., individual genes and gene groups) *of* differentiated neuroblastoma cells (e.g., *SH-SY5Y cells) infected by* HHV-6A or HHV-6B *and compare transcriptome* responses of roseolovirus-infected cells to uninfected controls. Roseolovirus inocula are administered at different multiplicities of infection (MOI) to assess how increasing MOI impacts the magnitude of up- or down-regulation of gene expression. Likewise, cytopathic effects (CPE) will be monitored to map -omics to physiology in these experimental infections. Distinct time-points post-infection are selected to monitor changes in gene expression during different stages of infection. Although there are limitations in interpreting in vitro data from differentiated neuroblastoma cells, these data provide a starting point for the identification of particular genes and groups of genes that may be dysregulated during roseolovirus infection of nerve cells.

Disclosures: A. Bajpai: None. **E. Bahramian:** None. **R. Ceballos:** None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.13/TT7

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH-NICHD (5T32HD007470-29)
NIH-NICHD (5T32HD007470-28)

Title: Defining molecular features of epileptic networks using intracranial sEEG-derived patient tissue

Authors: *J. HARRIS[1], O. PASTOR ALONSO[1], M. F. PAREDES[2];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]UCSF, San Francisco, CA

Abstract: Malformations of cortical development (MCD) are a leading cause of intractable pediatric epilepsy. However, approximately one third of patients with MCD lack clearly defined lesions using current neuroimaging technologies. In imaging negative patients, neuromonitoring with intracerebral stereoelectroencephalography (sEEG) can isolate seizure foci for future surgical resection. Patient material adhered to sEEG electrodes can be used to isolate DNA to identify somatic variants within the epileptic network not present in the germline. However, how these mutations impact the transcriptomic environments across the seizure network remains unknown. We evaluated patient tissue adhered to sEEG electrodes to identify genomic and transcriptomic changes within the seizure-onset (ictal) and non-ictal brain regions in a cohort of 12 pediatric patients with intractable, imaging negative focal epilepsy. This unique source of patient tissue can identify somatic mutations and generate biomarker libraries of target genes misregulated in the ictal versus non-ictal brain regions. Additionally, we observe cell type specific changes to the molecular pathways using single nucleus RNA sequencing on surgically resected tissue from a subset of these sEEG patients. In combination, we have identified molecular features of the ictal region using sEEG electrodes and resected tissue by correlating transcriptomic findings with local patterns of ictogentic activity.

Disclosures: J. Harris: None. **O. Pastor Alonso:** None. **M.F. Paredes:** None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.14/TT8

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant UM1 MH130994
NIH Grant U01 AG076791

Title: Human basal ganglia cell-type and cellular community organizations revealed by large-scale spatial transcriptomics mapping

Authors: ***X. XU**[1], B. BERACKEY[2], Z. TAN[1], G. WU[3], M. BEHRENS[4], B. REN[5], J. R. ECKER[6];
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Abstract: The large-scale and high-resolution spatial organization of basal ganglia cell types, cellular communities and gene expression gradients remains to be determined, and this poses a challenge for most spatial transcriptomics platforms which typically profile small pieces of the tissue. Here we leverage large multi-centimeter-scale capture capabilities and subcellular resolution of Stereo-seq (Spatio-Temporal Enhanced REsolution Omics-sequencing) and enhanced multiplexed error-robust fluorescence in situ hybridization (MERFISH+) to profile spatial gene expression of the human basal ganglia with 0.6-1.5 million cells per experiment to map multiple basal ganglia anatomical subdivisions using six human donor samples. Using the integrated dataset, we generated spatially resolved single-cell resolution maps that encompass multiple basal ganglia subregions. Stereo-seq and MERFISH+ approaches show excellent agreement in gene expression and cell-type analyses across matched adjacent sections. Single-cell level spatial transcriptomics maps identify major cell type clusters and reveal detailed molecular and cellular landscapes across basal ganglia subregions. We determined the precise spatial locations of >58 cell clusters annotated consistently with the Allen Institute's single-nucleus RNA-sequencing data, across the resolutions of five orders of magnitude from the multi-centimeter scale to the submicrometer scale. Within the dorsal striatum, the spatial locations of D1 medium spiny neurons (MSNs) and D2 MSNs differentiate the striosomes and matrix within the caudate and putamen. Spatial locations of ventral striatal D1 and D2 MSNs clearly identify the ventral striatum (nucleus accumbens) sub-region. We conducted spatial cellular community analysis on MERFISH+ data and uncovered three distinct communities/modules with distinct neuronal type compositions which are localized to the striosomes (composed of mainly D1 and D2 striosome MSNs), matrix (composed of mainly D1 and D2 matrix MSNs), and ventral striatum (mainly composed of ventral striatal D1 and D2 MSNs). In addition, the community analysis focused on oligodendrocytes show three large spatial tissue architectural modules that underlies the anatomical organization of the caudate nucleus, putamen, and nucleus accumbens. Interestingly the large spatial tissue architecture is correlated with multiple gene expression gradient maps obtained from Stereo-seq, establishing the link between molecular gene expression, cell types and spatial modular structures in the human basal ganglia.

Disclosures: X. Xu: None. **B. Berackey:** None. **Z. Tan:** None. **G. Wu:** None. **M. Behrens:** None. **B. Ren:** None. **J.R. Ecker:** None.

Poster

PSTR417: Spatial and Multi-omics II

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Program #/Poster #: PSTR417.15/TT9

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant UM1MH130981

Title: A cross-species spatial transcriptomic atlas of the human and non-human primate basal ganglia

Authors: ***M. HEWITT**[1], M. A. TURNER[1], S. C. SEEMAN[1], B. R. LONG[1], J. L. CLOSE[1], N. JOHANSEN[1], Y. FU[1], T. BAKKEN[1], R. D. HODGE[1], J. QUON[1], M. KUNST[1], M. HUANG[1], L. NG[1], J. ARIZA TORRES[1], D. MCMILLEN[1], F. YAZDANIBANAFSHEDARAGH[2], D. C. VAN ESSEN[3], W. FREIWALD[2], L. KRUSE[1], J. WATERS[1], T. JARSKY[1], C. D. KEENE[4], F. M. KRIENEN[5], H. ZENG[1], E. LEIN[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Rockefeller Univ., New York, NY; [3]Neurosci., Washington Univ. Sch. Med., St Louis, MO; [4]Pathology, Univ. of Washington, Seattle, WA; [5]Neurosci., Princeton Univ., Princeton, NJ

Abstract: The basal ganglia are a group of subcortical structures involved in motivation, action selection, and a variety of other functions. Though previous studies have shown complex patterns of gene expression in individual basal ganglia structures, it remains unclear whether these molecular and spatial "rules" of organization generalize across species and regions. In this study, we created a cellular-resolution, spatial transcriptomic atlas of the basal ganglia in three primate species: human, rhesus macaque, and common marmoset. We sampled the canonical basal ganglia structures - striatum, globus pallidus, subthalamic nucleus, and substantia nigra - across their full anatomical extent, resulting in a dataset of over 1 million cells in each species. By mapping these spatially resolved cells to a consensus, cross-species snRNA-seq cell type taxonomy, our atlas reveals conserved principles of molecular organization within and across basal ganglia structures. We find that basal ganglia structures are characterized both by discrete compartments of cell types and smooth gradients of gene expression. For instance, we find ventral striatum marker genes and cell types are present in the caudate tail, where transcriptomic diversity is organized along complex gene expression gradients that extend into the adjacent amygdalostriatal transition zone. In the internal globus pallidus, we observe distinct cell types organized into discrete core and shell compartments, a pattern that is conserved between primates and mouse. By integrating data across species and structures, our spatial transcriptomic atlas provides a key reference for future molecular and functional studies of the basal ganglia.

Disclosures: M. Hewitt: None. **M.A. Turner:** None. **S.C. Seeman:** None. **B.R. Long:** None. **J.L. Close:** None. **N. Johansen:** None. **Y. Fu:** None. **T. Bakken:** None. **R.D. Hodge:** None. **J. Quon:** None. **M. Kunst:** None. **M. Huang:** None. **L. Ng:** None. **J. Ariza Torres:** None. **D. McMillen:** None. **F. YazdaniBanafsheDaragh:** None. **D.C. Van Essen:** None. **W. Freiwald:** None. **L. Kruse:** None. **J. Waters:** None. **T. Jarsky:** None. **C.D. Keene:** None. **F.M. Krienen:** None. **H. Zeng:** None. **E. Lein:** None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.16/TT10

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant UM1MH130981

Title: Morphological and electrophysiological diversity and specialization in the non-human primate striatum

Authors: *X.-P. LIU[1], R. DALLEY[1], N. JOHANSEN[2], A. BUDZILLO[3], S. WALLING-BELL[1], J. A. MILLER[2], I. KAPEN[1], M. WIRTHLIN[4], S.-L. DING[5], T. BAKKEN[5], S. A. SORENSEN[8], T. JARSKY[6], G. TAMAS[10], N. W. GOUWENS[5], H. ZENG[1], E. LEIN[7], J. T. TING[7], B. R. LEE[9], B. E. KALMBACH[7];
[2]Human Cell Types, [3]Informatics and Data Sci., [4]Allen Inst. for Brain Sci., [1]Allen Inst. for Brain Sci., Seattle, WA; [6]Integrated Cell Physiol., [7]Human Cell Types, [5]Allen Inst. For Brain Sci., Seattle, WA; [9]Electrophysiology, [8]Allen Inst., Seattle, WA; [10]Univ. of Szeged, Szeged, Hungary

Abstract: The primate basal ganglia remain insufficiently understood, despite their critical role in reward processing, motor reinforcement learning, and numerous human disorders. Leveraging a high-resolution, cross-species Allen Institute taxonomy that reveals extensive neuronal diversity in the primate striatum, we applied the Patch-seq method to link transcriptomic identity to functional properties across anatomical divisions of the macaque and squirrel monkey striatum. This approach provides direct insight into cellular properties that constrain network function, such as dendritic morphology and intrinsic physiology. We highlight underappreciated diversity among medium spiny neurons (MSNs), with transcriptomic diversity being reflected in properties such as branching complexity, input resistance, and frequency filtering. Distinct MSN subtypes, including the less well-characterized D1D2 hybrids and a newly identified D2 hybrid group, showed unique combinations of morphological and electrophysiological features. Additionally, MSN properties varied within subclass in a graded manner over three-dimensional space aligned with functional domains. Interneuron groups exhibited even greater morpho-electric diversity, as well as systematic spatial variation. The recently identified, primate-specialized $TAC3^+$ interneurons resembled striatal fast-spiking interneurons in morphology and firing rate but likely differ in spiking mechanisms and show limited capacity to sustain high firing rates. Lastly, we identified numerous significant species-specific features distinguishing homologous rodent and primate striatal neurons, despite the evolutionary conservation of this structure and overall similarity between corresponding types. For example, cholinergic interneurons in macaques displayed markedly more elaborate morphologies than their murine counterparts, with increased arbor complexity and branching. Together, these findings offer insights into network behavior in primate striatum by shedding light on its cellular specializations and functional organization.

Disclosures: X. Liu: None. **R. Dalley:** None. **N. Johansen:** None. **A. Budzillo:** None. **S. Walling-Bell:** None. **J.A. Miller:** None. **I. Kapen:** None. **M. Wirthlin:** None. **S. Ding:** None. **T. Bakken:** None. **S.A. Sorensen:** None. **T. Jarsky:** None. **G. Tamas:** None. **N.W. Gouwens:** None. **H. Zeng:** None. **E. Lein:** None. **J.T. Ting:** None. **B.R. Lee:** None. **B.E. Kalmbach:** None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.17/TT11

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH Grant UM1MH130981

Title: A cellular-resolution spatial transcriptomic atlas of the marmoset subcortex

Authors: *M. A. TURNER[1], S. DAN[2], M. DEBERARDINE[2], L. CACERES[2], J. SCHEMBRI[2], Q. ZHANG[3,4], K. LEVANDOWSKI[3,4,5], D. MCMILLEN[1], N. MARTIN[1], N. VALERA CUEVAS[1], P. OLSEN[1], J. NAGRA[1], J. CAMPOS[1], M. VANNESS[1], J. WATERS[1], Z. C. JUNEAU[1], S. RANSFORD[1], S. D. HASTINGS[1], S. BARTA[1], A. RUIZ[1], J. ARIZA TORRES[1], G. FENG[4,3,5], H. ZENG[1], E. LEIN[1], F. M. KRIENEN[2], B. R. LONG[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [3]McGovern Inst. for Brain Res., [4]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [5]Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: The primate subcortex consists of over 200 heterogeneous nuclei that contribute to a vast array of brain functions. Subcortical nuclei are molecularly diverse and have complex topographical organization, but it is an ongoing effort to link the molecular identity of individual cells to existing anatomical parcellations of the brain. Leveraging the compact size of the common marmoset brain, we have generated the most comprehensive spatial transcriptomic atlas of primate subcortex to date, sampling the basal ganglia, basal forebrain, amygdala, hippocampal formation, thalamus, hypothalamus, and midbrain. We performed cellular-resolution spatial transcriptomics on intact hemispheres using the Xenium platform, imaging 5.6 million cells at ~200 µm-spaced intervals across the marmoset subcortex. Spatial cells were mapped to a snRNA-seq taxonomy of subcortical clusters with annotations derived from a consensus, cross-species basal ganglia taxonomy and existing human and mouse atlases. Transcriptomically defined cell types resolve discrete anatomical compartments throughout the subcortex, including individual nuclei of the amygdala, hypothalamus, and thalamus and subdivisions of the primate-expanded medial pulvinar. *De novo* analysis of spatially variable gene expression further reveals that many subcortical structures are organized along complex, multi-gene gradients and share gene expression programs with neighboring structures. By placing transcriptomic cell types in anatomical context, our spatial transcriptomic atlas provides a foundational resource for

understanding the molecular and functional organization of the diverse structures of the primate subcortex.

Disclosures: M.A. Turner: None. **S. Dan:** None. **M. DeBerardine:** None. **L. Caceres:** None. **J. Schembri:** None. **Q. Zhang:** None. **K. Levandowski:** None. **D. McMillen:** None. **N. Martin:** None. **N. Valera Cuevas:** None. **P. Olsen:** None. **J. Nagra:** None. **J. Campos:** None. **M. VanNess:** None. **J. Waters:** None. **Z.C. Juneau:** None. **S. Ransford:** None. **S.D. Hastings:** None. **S. Barta:** None. **A. Ruiz:** None. **J. Ariza Torres:** None. **G. Feng:** None. **H. Zeng:** None. **E. Lein:** None. **F.M. Krienen:** None. **B.R. Long:** None.

Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.18/TT12

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Co-variation between molecular expression, physiology, morphology and spatial location in mouse basal ganglia neurons

Authors: *A. BUDZILLO, M. MALLORY, R. DALLEY, C. LEE, X.-P. LIU, X. CI, S. WALLING-BELL, R. MANN, N. JOHANSEN, B. TASIC, H. ZENG, B. E. KALMBACH, Z. YAO, T. JARSKY, S. A. SORENSEN, B. R. LEE, N. W. GOUWENS;
Allen Inst., Seattle, WA

Abstract: The basal ganglia (BG) are a topographically organized, interconnected set of structures that are pivotal for regulating volitional movement and other aspects of behavior. Considerable progress has been made in describing the diverse cellular landscape within BG structures in rodents and primates. Taxonomies of transcriptomically-defined cell types (t-types) have revealed both fine-grained distinctions between neurons in these structures as well as continuous transcriptomic variation across similar t-types, which can be related to location within a structure. However, it remains unclear to what extent the spatial and transcriptomic variation correspond with each other and with other cellular properties, such as electrophysiological and morphological characteristics. Therefore, we performed Patch-seq experiments on > 1000 neurons in mouse brain slices from BG to provide an integrated view of the co-variation between molecular expression, physiological and morphological properties in single neurons in the context of their spatial location. We found that the phenotypes of most medium spiny neurons (MSNs) across the dorsal and ventral striatum follow a molecularly-defined spatial gradient with faster action potential kinetics and more extensive dendritic arborizations at its dorsolateral extent. Furthermore, we found that this relationship between the transcriptomic gradient and electrophysiological properties appears to be conserved across species by comparing to Patch-seq data from macaque. We observed that certain MSNs exhibit a unique phenotype apart from the broader continuum; for example, we measured comparatively higher excitability in a recently reported novel Htr7+ D2 MSN t-type. We also characterized how

the properties of striatal interneurons vary in the context of the MSN gradient. In addition, we made Patch-seq recordings in the globus pallidus, subthalamic nucleus, and substantia nigra and used the multimodal data to draw correspondences between molecularly-defined t-types and previously described phenotypes to refine our descriptions of cell types in these structures. Finally, we examined how co-variation across modalities could be linked across different stages of the BG circuit by comparing Patch-seq neurons to complete reconstructed whole-neuron morphologies and the topography of their interareal projections. These findings provide key validation of t-types in mouse BG, linking them to spatial localization and phenotypic differences on a single-cell level, and improve our understanding of the cell type landscape in topographically organized circuits across the brain.

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Poster

PSTR417: Spatial and Multi-omics II

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Program #/Poster #: PSTR417.19/TT13

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: 1UM1MH130981-01

Title: Multimodal Profiling of Basal Ganglia Astrocytes within a Cross-species Consensus Atlas

Authors: *Y. FU[1], N. JOHANSEN[5], J. L. CLOSE[6], M. HEWITT[7], M. A. TURNER[7], B. R. LONG[9], S. C. SEEMAN[7], X.-P. LIU[7], M. WIRTHLIN[8], W. DING[13], S. DAN[14], M. DEBERARDINE[14], I. KAPEN[7], A. GARCIA[2], D. BERTAGNOLLI[1], A. A. BHANDIWAD[7], A. BUDZILLO[3], L. CACERES[14], A. CHAKKA[4], R. CHAKRABARTY[1], S. DANIEL[1], S.-L. DING[9], R. FERRER[7], J. GOLDY[10], J. R. ECKER[15], K. SMITH[7], F. M. KRIENEN[16], R. D. HODGE[11], E. LEIN[12], T. BAKKEN[9];
[2]Human Cell Types, [3]Informatics and Data Sci., [4]Mol. Biol., [1]Allen Inst., Seattle, WA; [5]Human Cell Types, Allen Inst. for Brain Sci., Seattle, WA; [6]Imaging, Allen Inst. for Brain Sci., SEATTLE, WA; [8]Allen Inst. for Brain Sci., [7]Allen Inst. for Brain Sci., Seattle, WA; [10]BiCore, [11]Cell Types Program, [12]Human Cell Types, [9]Allen Inst. For Brain Sci., Seattle, WA; [13]GALE, Salk Inst. for Biol. Studies, San Diego, CA; [14]Princeton Neurosci. Inst., Princeton, NJ; [15]PBIO-E, Salk Inst., La Jolla, CA; [16]Neurosci., Princeton Univ., Princeton, NJ

Abstract: The basal ganglia (BG) are evolutionarily conserved subcortical brain structures that are essential for motor control, learning, emotion, and cognition, and are implicated in neurological and psychiatric disorders. Yet, a unified molecular taxonomy of BG cell types across species is lacking. Here, we present a multi-omic consensus atlas comprising over two

million nuclei from human, macaque, and marmoset spanning eight major structures of the BG circuit. By integrating single-nucleus transcriptomics, chromatin accessibility, DNA methylation, 3D chromatin conformation, spatial transcriptomics, morphology, and electrophysiology, we achieved multimodal characterization of BG cell types. Cross species alignment reveals 60 homologous cell types conserved over 80 million years of mammalian evolution, while also highlighting species-specific innovations. As a key example of human glial specialization, we construct a detailed multimodal atlas of BG astrocytes. In human BG, we identify 21 transcriptionally distinct astrocyte subtypes, which resolve into three major groups—striatal gray matter, non-striatal gray matter, and white matter—each defined by differential expression of neurotransmitter transporters and synapse associated genes. These molecular patterns reflect circuit input architecture rather than local neuronal identity, revealing a circuit aligned mode of astrocyte specialization. Within the striatum, we identify a novel dorsal and ventral spatial segregation of astrocytes supported by methylation, chromatin conformation, and histone data. Comparative analysis across primates identifies a human-specific astrocyte subtype enriched in the substantia nigra, marked by elevated gliotransmission signatures and expression of genes linked to Parkinson's disease vulnerability. Together, this multi-species, multi-omic BG atlas establishes a foundational resource for understanding the molecular logic of cell identity, circuit function, and evolutionary divergence across both neuronal and glial lineages.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.20/TT14

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: 1UM1MH130981-01

Title: Multimodal Properties of Glutamatergic Neurons in the Human Neocortex

Authors: *R. DALLEY[1], S. WALLING-BELL[1], X.-P. LIU[1], F. WALEBOER[2], J. A. MILLER[1], T. JARSKY[1], N. GORIOUNOVA[2], C. P. DE KOCK[2], G. TAMAS[3], H. D. MANSVELDER[2], N. W. GOUWENS[1], J. T. TING[1], H. ZENG[1], E. LEIN[1], B. E. KALMBACH[1], S. A. SORENSEN[1], B. R. LEE[1];
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Abstract: Understanding the properties of cell types in the human brain is vital for advancing our knowledge of human health and cognition. Single-nucleus transcriptomic taxonomies have become the standard for characterizing cell types, their diversity and potential vulnerabilities to disease. Defining their multimodal characteristics is crucial for linking molecular identity to function, yet such definitions remain lacking for most transcriptomically-defined human cell types. To address this, we combine Patch-seq— a method for collecting Morphology, Electrophysiology, and Transcriptomic data from a single neuron— with spatial transcriptomics, to provide a comprehensive description of human cortical neurons across nine glutamatergic subclasses, encompassing 42 transcriptomically defined cell types. We integrate data from 1486 transcriptomic, 986 electrophysiological, and 320 morphological recordings across all layers of the cortex to refine existing transcriptomic classifications based on multimodal properties. We observe large differences in apical dendritic morphology across cell types, including notable diversity in layer 6, which likely reflects functional differences. Cross-species comparisons show a surprisingly greater number of dendritic branches in macaque neurons compared to homologous human cell types. Comparisons of neurons from different cortical regions reveal variations in electrophysiological and morphological properties for the same transcriptomically defined cell types. Finally, we characterize the electrophysiological properties of infragranular cell types vulnerable to Alzheimer's disease (AD), establishing a foundational understanding of key mechanisms that may underlie AD development and progression. This dataset provides the scientific community with a unified reference to explore and accelerate our understanding of the human brain.

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Poster

PSTR417: Spatial and Multi-omics II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.21/TT15

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Title: Developmental Regulation of Morphoelectric Properties of Human Cortical Neurons

Authors: K. NASIROVA[1], R. DALLEY[2], A. BUDZILLO[3], S. WALLING-BELL[2], S. SAWCHUK[4], N. DEE[2], H. GOLDSTEIN[5], J. HAUPTMAN[6], J. G. OJEMANN[7], N. W. GOUWENS[8], T. JARSKY[9], E. LEIN[10], J. T. TING[10], H. ZENG[2], S. A. SORENSEN[1], B. E. KALMBACH[10], ***B. R. LEE**[1];
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Abstract: The human brain undergoes a prolonged postnatal development that spans distinct phases ranging from infancy, toddler, childhood, adolescence, and into adulthood. The complex interplay between cell types, from neurogenesis through synaptic pruning in mature circuits, involves diverse neuron types with each playing critical roles in the cortical circuit. Due to recent advances in single-cell RNA-sequencing, we are beginning to understand the molecular framework of neurons through these key stages of development. However, a deeper understanding of the corresponding phenotypic properties and circuit dynamics is lacking. Intrinsic membrane properties are crucial for defining circuit dynamics and regulation of synaptic plasticity and it is hypothesized that these may change along the development trajectory. To provide insight into how functionally related properties like local axonal/dendritic morphology and electrophysiology change in distinct cell types across human postnatal development we performed Patch-seq experiments in *ex vivo* brain slices obtained from neurosurgical tissue from patients 6 weeks old through adulthood. To aid in the targeting of specific cell types we applied enhancer-based adeno-associated viral vectors in organotypic slice cultures prepared from these tissues. We identified key functional properties, such as action potential width and firing and dendritic arborization that change in glutamatergic and GABAergic transcriptomic types. Finally, we identified relevant genes that are likely responsible regulating these processes during neuronal development. Gaining a deeper understanding of the phenotypic properties of cortical neurons during development will provide a critical insight into neural circuitry and connectivity, cognitive function and disease.

Disclosures: K. Nasirova: None. **R. Dalley:** None. **A. Budzillo:** None. **S. Walling-Bell:** None. **S. Sawchuk:** None. **N. Dee:** None. **H. Goldstein:** None. **J. Hauptman:** None. **J.G. Ojemann:** None. **N.W. Gouwens:** None. **T. Jarsky:** None. **E. Lein:** None. **J.T. Ting:** None. **H. Zeng:** None. **S.A. Sorensen:** None. **B.E. Kalmbach:** None. **B.R. Lee:** None.

Poster

PSTR417: Spatial and Multi-omics II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.22/TT16

Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH UM1MH130981

Title: A transcriptomic and regulatory atlas of the marmoset subcortex

Authors: *S. DAN[1], M. A. TURNER[2], M. DEBERARDINE[1], N. WANG[1], V. NIETO CABALLERO[1], L. CACERES[1], J. SCHEMBRI[1], Q. ZHANG[3], K. LEVANDOWSKI[4], B. ROSEN[5], D. MCMILLEN[2], N. MARTIN[2], N. VALERA CUEVAS[2], P. OLSEN[2], J. NAGRA[2], J. CAMPOS[2], M. VANNESS[2], J. WATERS[2], S. RANSFORD[2], Z. C. JUNEAU[2], S. BARTA[2], A. RUIZ[2], J. HARWELL[5], T. HAYASHI[6], D. C. VAN ESSEN[7], E. LEIN[2], H. ZENG[2], G. FENG[3], B. R. LONG[2], F. M. KRIENEN[1];
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Abstract: Understanding the cellular composition and gene regulatory landscape of the human brain remains a central challenge in neuroscience. Although whole-brain transcriptomic atlases exist for the mouse, the size and complexity of human and nonhuman primate brains have limited similarly comprehensive efforts in primates. By exploiting the compact neuroanatomy of the marmoset, a New World monkey, we generated paired RNA and ATAC single nucleus sequencing of ~700,000 nuclei sampled across densely tiled subcortical regions obtained from brains with atlas-registered structural and functional MRI. Single cell multiome data were mapped to a whole-subcortex marmoset spatial transcriptomics atlas for anatomical annotations. This resource constitutes the most extensive transcriptomic and regulatory atlas of the primate subcortex to date, covering the basal ganglia and all regions of the central nervous system outside of the neocortex and cerebellum. Cross-species alignment to an integrated basal ganglia taxonomy and existing mouse and human atlases enabled interoperable cell type annotations and highlighted divergent features between primates and mice. Subcortical neurons exhibited strong regional identity but varied in heterogeneity across structures. For example, hypothalamic neurons shared greater transcriptional similarity with midbrain populations than with neighboring forebrain domains. These transcriptional relationships were further reflected in their underlying regulatory programs. In the basal ganglia, chromatin accessibility and gene expression hierarchies are largely aligned, but not perfectly concordant, suggesting that transcriptional identity is often (but not always) mirrored by cis-regulatory architecture. We combined gene regulatory network inference with spatial mapping to identify regional patterns of both gene expression and cis-regulatory element activity, revealing anatomical- and lineage-associated determinants of cellular identity. This integrated multi-omics atlas of the marmoset subcortex provides a molecular framework for primate subcortical organization to understand circuits and functions.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

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Topic: J.01. Molecular, Biochemical, and Genetic Techniques

Support: NHGRI R43 013071
NHGRI R44 013071
NIDA R43057749

Title: Chromatin profiling from formalin-fixed paraffin-embedded (FFPE) samples to advance neuroscience research

Authors: *A. ALCALA[1], E. BRILL[1], V. UDAYAKUMARAN NAIR SUNITHA KUMARY[1], B. J. VENTERS[1], J. SCHUELER[2], M. KEOGH[1];
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Abstract: Understanding the epigenetic landscape is increasingly important for neuroscience research, offering insights into brain development, function, and mechanisms underlying neurological disorders. Cell function and fate are regulated by epigenomic features, including histone post-translational modifications (PTMs), transcription factors, and other chromatin proteins. Mapping the location of these features provides a powerful approach to study epigenomic mechanisms underlying health and disease. Formalin-fixed paraffin-embedded (FFPE) tissues are the gold-standard for the preserving clinical and neurological samples. However, using FFPE tissues for chromatin mapping assays has been technically challenging. For instance, heavy cross-linking makes FFPEs unsuitable for chromatin accessibility profiling (ATAC-seq), while the low chromatin yields extracted from FFPEs make certain mapping assays (ChIP-seq) impractical. Combined, these difficulties have precluded widespread application of FFPE tissues for epigenomics research. To better leverage chromatin profiling for biomedical research, we are developing a modified CUT&Tag workflow that is compatible with FFPE samples (CUT&Tag-FFPE). CUT&Tag (Cleavage Under Targets and Tagmentation) is an immunotethering-based strategy that uses pAG-Tn5 to selectively cleave and ligate sequencing adapters at antibody-bound chromatin. The assay uses intact cells or nuclei bound to a solid support, enabling a highly streamlined, 2-day workflow. Compared to ChIP-seq, CUT&Tag requires 10-1,000x fewer cells, 5-10x less sequencing, and is scalable for large studies. Its sensitivity makes it especially well-suited for neurobiological applications that require limited or precious samples. We have optimized upstream sample processing steps for efficient extraction and permeabilization of nuclei from FFPE tissue. We have applied these modifications in heavily fixed cell lines and liver and colon FFPE tissues to successfully map RNA polymerase II (RNAPII), the polymerase that transcribes mRNA and non-coding RNA. RNAPII profiling data correlates with transcriptomic data from native samples, providing a direct readout of transcription. Future work will validate additional chromatin targets using matched FFPE and fresh-frozen neurological samples. We are also optimizing workflows to pair with downstream single-cell and spatial platforms. We envision that CUT&Tag-FFPE, and the associated automated assay service we are developing for large scale studies, will accelerate the discovery of epigenetic drug targets and biomarkers to advance biomedical research.

Disclosures: A. Alcala: A. Employment/Salary (full or part-time): EpiCypher. **E. Brill:** A. Employment/Salary (full or part-time): EpiCypher. **V. Udayakumaran Nair Sunitha Kumary:** A. Employment/Salary (full or part-time): EpiCypher. 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. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpiCypher. **J. Schueler:** A. Employment/Salary

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.24/TT18

Topic: J.02. Systems Biology and Bioinformatics

Support: NIH Grant R01MH096906
NIH Grant U24NS140384

Title: Meta-analytic functional decoding of the cortical terminations of the 45 major white matter tracts

Authors: *K. L. RAY[1], S. KIM[3], J. PERAZA[4], S. GUO[1], A. R. LAIRD[5], F. PESTILLI[2]; [2]Psychology, [1]The Univ. of Texas at Austin, Austin, TX; [3]Psychology, Univ. of Texas at Austin, Austin, TX; [5]Physics, [4]Florida Intl. Univ., Miami, FL

Abstract: White matter tracts (WMTs) support brain function and behavior by connecting distal cortical regions. Over 40 major WMTs can now be routinely mapped, but their cortical terminations and links to cognitive functions remain poorly understood. Here, we leverage Human Connectome Project (HCP) data, a reproducible WM mapping platform, and meta-analytic methods to functionally characterize WMT cortical termination maps (CTMs).Diffusion MRI data from 1,062 HCP subjects were processed using brainlife.io to create whole-brain tractograms. Tractograms were used to map WM termination points of 45 major WMTs on the cortical surface. CTMs were transformed to MNI space and averaged to create a cortical termination density atlas. Independent of WM analyses, a Latent Dirichlet allocation (LDA)-based decoder trained on 13,459 functional MRI studies provided 104 functional topics and their associated brain activation coordinates. CTMs were decoded using the LDA model to link WM terminations to cognitive functions. Hierarchical clustering analysis (HCA) then grouped WMTs based on these functional associations.The cognitive functions linked with the CTMs of 4 exemplary tracts used for validation are detailed. The vertical occipital fasciculus (VOF) showed strong associations with visual processing. The Inferior Longitudinal Fasciculus (ILF) had posterior terminations in the occipital lobe linked to visual functions (e.g., motion detection, saccades, object recognition), while anterior terminations in the temporal lobe were associated with auditory processing (e.g., speech and music perception) and higher-order cognition (e.g., sentence comprehension), highlighting the ILF's role in integrating visual, auditory, and semantic information. The arcuate fasciculus (AF), connecting Broca's and Wernicke's areas, was tied to language production and comprehension. The corticospinal tract (CST) was linked to motor functions like voluntary movement and fine motor control.HCA revealed 5 clusters: Cluster 1 (language-related tracts like the AF, associated with semantic and syntactic

processing), Cluster 2 (tracts related to self-referential processing and the default mode network), Cluster 3 (motor-related tracts, including the CST), Cluster 4 (visual-processing tracts in the occipital lobe), and Cluster 5 (tracts tied to task performance and motor control in the precentral and postcentral gyri).This work presents an atlas of CTMs with quantitative meta-analytic functional decoding. HCA identified 5 WMT clusters based on functional similarity, offering insights into the diverse processes and networks supported by WMTs and their role in brain function.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.25/UU1

Topic: J.02. Systems Biology and Bioinformatics

Title: Whole-body mapping of peripheral nerve and immune alterations in obesity using 3D imaging and deep learning

Authors: *D. KALTENECKER[1], I. HORVATH[2], R. AL-MASKARI[4], Y. CHEN[2], M. I. TODOROV[5], A. M. ERTURK[3];
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Abstract: Many diseases, including obesity, affect multiple organ systems and are able to influence both the nervous and immune systems. However, tools for comprehensive mapping of these effects across the whole body have been limited. We developed *MouseMapper*, a deep learning-based pipeline integrated with tissue clearing and light-sheet microscopy, enabling 3D quantification of changes in peripheral nerve and immune cell changes across the entire mouse body. In a high-fat diet (HFD) mouse model, we used *MouseMapper* to identify obesity-associated remodeling of peripheral nerves, including marked structural changes in the infraorbital nerve, a part of the trigeminal system critical for facial sensation. These changes were associated with impaired whisker-evoked responses and proteomic alterations in the trigeminal ganglion, including dysregulation of pathways involved in axon guidance, cytoskeletal dynamics and inflammation. Additionally, we observed widespread, tissue-specific

shifts in immune cell distributions, highlighting the systemic nature of obesity-induced inflammation. Our study introduces *MouseMapper* as a versatile tool for whole-body neuroimmune analysis and reveals new insights into the neurobiological sequelae of obesity.

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Poster

PSTR417: Spatial and Multi-omics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR417.26/UU2

Topic: J.02. Systems Biology and Bioinformatics

Title: Comparative spatial analyses of the tumor immune landscape in mouse models of glioblastoma using the PhenoCode™ Mouse Neuro Panel and customized markers

Authors: Y. PEYMANFAR, M. POULIN, R. MIHANI, ***F. ROLAND**;
Akoya Biosci., Marlborough, MA

Abstract: Glioblastoma (GBM) presents a highly immunosuppressive and spatially heterogeneous tumor microenvironment (TME), posing major challenges for therapeutic intervention. To gain deeper insights into the cellular architecture of GBM, we applied high-plex spatial proteomics to characterize the immune and neural landscapes in wild-type and GL261 glioma-bearing mouse brains. Spatial imaging was performed using the PhenoCycler®-Fusion platform, leveraging a pre-commercial version of Akoya's PhenoCode™ Mouse Neuro Panel, supplemented with customized markers to capture additional immune and tumor-relevant biology. The full panel enabled simultaneous visualization of over 40 markers, including mature and immature neurons (NeuN, MAP2, DCX), astrocytes (GFAP), oligodendrocytes (MBP), microglia and macrophages (Iba1, CD68, F4/80), T cells (CD3, CD8, PD1), vascular markers (CD31), and indicators of proliferation and DNA damage (Ki67, H2AX). Whole-slide spatial analysis revealed substantial remodeling of the TME in the GL261 model, including microglial activation, T cell infiltration, and disruption of neurovascular structure and astrocytic networks. Dimensionality reduction and clustering revealed distinct spatial niches and immune neighborhoods, providing insight into how the GBM microenvironment evolves across tissue compartments. This study demonstrates the utility of high-plex spatial proteomics for dissecting complex tumor ecosystems and highlights the potential of the developing PhenoCode™ Mouse Neuro Panel as a powerful tool for preclinical neuro-oncology research.

Disclosures: Y. Peymanfar: A. Employment/Salary (full or part-time): Akoya Biosciences. **M. Poulin:** A. Employment/Salary (full or part-time): Akoya Biosciences. **R. Mihani:** A. Employment/Salary (full or part-time): Akoya Biosciences. **F. Roland:** A. Employment/Salary (full or part-time): Akoya Biosciences.

Poster

PSTR418: Connectomics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.01/UU3

Topic: J.03. Anatomical Methods

Support: The University of Notre Dame College of Science Summer Research Fellowship (ZMK)
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The University of Notre Dame (CJS)
The SMART foundation (CJS)
NIH Grant DP2NS117177 (CJS)

Title: An ultrastructural map of a spinal sensorimotor circuit reveals the potential of astroglial modulation

Authors: *Z. M. KOH[1,2], R. AVALOS[1,2], J. HAMMER[1,2], K. CHAU[1,2], S. E. LIGHT[1,2], A. DOLOJAN[1,2], M. JANUSZEWSKI[3], F. SVARA[4], C. J. SMITH[1,2];
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Abstract: Information flow through circuits is dictated by the precise connectivity of neurons and glia. While a single astrocyte can contact many synapses, how glial-synaptic interactions are arranged within a single circuit to impact information flow remains understudied. Here, we use the local spinal sensorimotor circuit in zebrafish as a model to understand how neurons and astroglia are connected in a vertebrate circuit. With semi-automated cellular reconstructions and automated approaches to map all the synaptic connections, we identified the precise synaptic connections of the local sensorimotor circuit, from dorsal root ganglia neurons to spinal interneurons and finally to motor neurons. This revealed a complex network of interneurons that interact in the local sensorimotor circuit. We then mapped the glial processes within tripartite synapses in the circuit. We demonstrate that tripartite synapses are equally distributed across the circuit, supporting the idea that glia can modulate information flow through the circuit at different levels. We show that multiple astroglia, including bona fide astrocytes, contact synapses within a single sensory neuron's circuit and that each of these astroglia can contact multiple parts of the circuit. This detailed map reveals an extensive network of connected neurons and astroglia that process sensory stimuli in a vertebrate. We then utilized this ultrastructural map to model how synaptic thresholding and glial modulation could alter information flow in circuits. We validated this circuit map with GCaMP6s imaging of dorsal root ganglia, spinal neurons and astroglia. This work provides a foundational resource detailing the ultrastructural organization of neurons and glia in a vertebrate circuit, offering insights in how glia could influence information flow in complex neural networks.

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Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ariadne.ai ag. **C.J. Smith:** None.

Poster

PSTR418: Connectomics II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR418.02/UU4

Topic: J.03. Anatomical Methods

Support: Austrian Science Fund FWF 35614

Title: Tissue Clearing of whole pig brains

Authors: *H.-U. DODT[1], M. PICKART[2], L. BOCANEGRA GOMEZ[2], H. SAMI[2,3];
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Abstract: After the presentation of the first cleared mouse brain (Dodt et al., SfN 2005) 20 years ago and its subsequent publication[2], clearing of brains of different specimens has become a widely used approach for the investigation of different neurobiological questions. The size of the investigated specimens has also increased. As a first step towards a cleared human brain with sufficient transparency for 3D recording as a whole we have developed a technology to clear whole pig brains with high transparency. To achieve this the key technology was the application of a chemical reaction for dehydration instead of the usual stepwise substitution of water with organic solutions. We had used this chemical reaction before to clear cm large pieces of breast cancer which are notoriously hard to clear due to their high cell density[2]. Key to high transparency in organic tissue clearing is always the complete elimination of water in the sample before submersion in a clearing solution like BABB (Benzylalkohol and Benzylbenzoat). Also complete delipidation must be performed to achieve good transparency even in the lipid rich brain parts like white matter and basal ganglia. We believe that the development of tissue clearing technology is by far not at its end and will be the decisive technology to obtain a complete transparent human brain for recording with light sheet microscopy.[1] Dodt HU et al., Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. Nat Methods. 2007, 4: 331-6. [2] Sabdyusheva Litschauer I et al., 3D histopathology of human tumours by fast clearing and ultramicroscopy. Sci Rep. 2020, 19: 17619.

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Poster

PSTR418: Connectomics II

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Topic: J.03. Anatomical Methods

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Title: Pencils Down, Everyone: Addressing the question of when to stop proofreading an electron microscopy image volume

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Abstract: In the past decade, the field of nanoscale connectomics has seen the development of advanced data generation pipelines that are creating petascale image volumes and associated dense segmentation annotations. While the performance of automated machine segmentation continues to improve, these process flows still generate data with reconstruction errors, such as merged and split neuron segments, that need to be corrected to ensure dataset integrity. Based on the recent Wellcome Trust "Scaling Up Connectomics" report, the most expensive and time-consuming step in the connectomics generation pipeline is proofreading. Thus, workflows for proofreading these datasets to identify and correct these errors, such as automated and manual neurite extension and pruning, must scale up massively to keep pace with dataset growth. While metrics have been developed to assess reconstruction accuracy, there are limited approaches beyond neuroanatomical expert assessment to assert when the dataset has achieved a sufficient level of quality for downstream science. To address this problem, we present an inexpensive and rapid method accompanying a proofreading workflow to incrementally evaluate the quality of data products such as connectomes (synaptic graphs) and contactomes (contact graphs). This approach assesses the asymptotic changes in connectivity and cell-cell contacts as proofreading edits are applied, as early, "easy" edits generally provide the greatest impact and later "hard" edits provide a diminishing return on effort and resources. Our method seeks to reduce the uncertainty associated with the planning and prioritization of proofreading activities toward producing a quality dataset. Using cloud resources, we subdivide computation into thousands of individual tasks and compute a single connectome in mere seconds at a total cost of $4 in cloud resources. We then employ cloud-native services such as chunked storage, on-demand ephemeral compute, and serverless databases, to execute and stitch these results rapidly. Finally, we repeat this rapid and inexpensive approach at several proofreading checkpoints and combine the results for downstream completeness analysis. We demonstrate the usefulness of this approach in a zebrafish spinal cord EM volumetric dataset of size 74 x 74 x 207 um [1]. These efforts will enable nanoscale connectome generators to forecast the amount of proofreading necessary for future datasets and democratize access to workflows for generating downstream data products at a speed and scale previously unachievable via conventional compute resources. [1] https://www.biorxiv.org/content/10.1101/2025.03.05.641432v1.full.pdf

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Poster

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Title: Multiscale Fluorescence Imaging of Human Brainstem Myeloarchitectonics

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Abstract: Characterizing the three-dimensional (3D) myelo-architecture of the human brain is a challenging task due to the complexity of its biological composition. The advent of sophisticated 3D imaging modalities, such as light sheet fluorescence microscopy (LSFM) and two-photon fluorescence microscopy (TPFM) has marked significant progress in neuroscience by providing new insights into the intricacies of human brain architecture. The combination of LSFM and TPFM with optical tissue clearing protocols, expansion methods, and advanced bioimage analysis allows for the study of the myeloarchitectonics of 3D volumetric areas of the human brain at unprecedented resolution. Here, we employed a combination of the aforementioned techniques to obtain a detailed map of the orientation of fibers at multiple resolutions, overcoming the optical limit, creating a complete atlas and connectome map in 3D of a human brainstem. Specifically, we employed the SHORT clearing method in combination with an advanced double-view LSFM to perform a volumetric study of the human brainstem, then, a specific area was selected and expanded four times using an innovative method named SHORT-Ex and acquired with a custom made TPFM. To automatically analyze the images and determine the 3D fiber orientation we developed the automatic tool called Foa3D. The work presented here demonstrates the feasibility of obtaining a multiscale 3D reconstruction of the human brain fiber architecture at multiple resolution, paving the way for the creation of a comprehensive atlas of the connectomes. This would provide a deep understanding of brain function and anatomical information useful for deciphering neuronal patterns in both healthy and diseased conditions.

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Poster

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Program #/Poster #: PSTR418.05/UU7

Topic: J.03. Anatomical Methods

Title: Anatomical connectivity between brainstem, cerebellum, thalamus and basal ganglia using diffusion weighted magnetic resonance imaging.

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Abstract: Diffusion Weighted Imaging (DWI) has been widely used to study the spatial organization of white matter pathways connecting different brain regions, yet most research has focused on cortico-cortical bundles, often neglecting corticofugal and non-cerebral fibers such as those in brainstem and other subcortical structures. This study evaluates the feasibility of using DWI to map anatomical connectivity between brainstem nuclei, deep cerebellar nuclei, cerebellar lobules, basal ganglia, subthalamic, thalamic and intralaminar nuclei. DWI data from 21 healthy subjects were acquired at 3 Tesla MRI with 2 mm isotropic resolution using a multishell protocol (132 directions: 64 at b = 2000 s/mm2, 64 at b = 1000 s/mm2, and 4 at b ≈ 0 s/mm2), and preprocessed for noise reduction (Low-Pass Condition Adaptation, LPCA), field inhomogeneity, and eddy current correction (MRtrix3). Parcellation was performed using Brainstem Navigator, SUIT, CERES, Morel atlas, and FreeSurfer. A modified mask, including brainstem and deep cerebellar nuclei within subcortical gray matter, was used for probabilistic Anatomically Constrained Tractography (10 million streamlines and default parameters from Mrtrix3). The resulting tractogram was filtered using SIFT to 1 million streamlines. Connectivity matrices were generated and averaged across subjects, producing a fully connected adjacency matrix. The natural logarithm of one plus the connectivity matrix was obtained and thresholded to one standard deviation from the mean (≥3.73); this revealed connectivity patterns consistent with classical neuroanatomy, including: (1) fibers linking the substantia nigra, caudate, pallidum, subthalamic nucleus, and ventrolateral thalamus (direct and indirect basal ganglia pathways); (2) vestibulocerebellar and olivocerebellar projections; (3) strong connectivity between the mesencephalic reticular formation and intralaminar thalamic nuclei; and (4) less commonly described pathways such as those connecting the parabigeminal nucleus with the inferior colliculus, implicated in visual attention modulation. These findings demonstrate that DWI is a viable method for investigating subcortical and brainstem connectivity, revealing anatomical pathways consistent with those identified using traditional tracer techniques.

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Poster

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Program #/Poster #: PSTR418.06/UU8

Topic: J.03. Anatomical Methods

Support: SSTF-BA2401-06

Title: Feedforward, feedback, and bilateral visual pathways in Drosophila revealed by a whole-brain connectome

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Abstract: The visual system of *Drosophila melanogaster* comprises approximately 60% of its brain cells, underscoring the central role of vision in this model organism. While some visual neurons have been characterized through targeted experiments, the majority remain uncharacterized. To investigate their roles and uncover structural principles of the visual system, we analyzed the synapse-level whole-brain connectome (FlyWire), focusing on neurons connecting an optic lobe (OL) with other brain regions. We classified these neurons into three types based on the distribution of input and output synapses: feedforward (OL to central brain, 3,176 cells), feedback (central brain to OL, 325 cells), and bilateral (between left and right OLs, 106 cells). We found that many feedforward neurons act as bottlenecks, integrating inputs across layers and columns and projecting to diverse central brain targets (median: 57 input / 45 output partner cells; 16/20 for OL intrinsic neurons and 29/27 for central brain neurons). Clustering feedforward neurons by postsynaptic targets revealed 30 groups and 7 meta-clusters. Some clusters contained neurons with similar visual features (e.g., LC12, LC17), while others grouped functionally distinct types (e.g., LC15, LC21), suggesting varying degrees of feature integration. Feedback neurons received ~40 ± 25% of inputs from feedforward neurons and ~25 ± 21% from ascending neurons. Their outputs within the OL overlapped with input layers of associated central brain feedforward partners, forming reciprocal loops (43 ± 24% ipsilateral, 20 ± 22% contralateral). We also analyzed bilateral neurons for spatial coverage across both eyes. Many, including LC14, exhibited significant overlap in receptive fields (e.g., LC14a1: 33 ± 20%; LC14a2: 39 ± 28%; LC14b: 24 ± 13%), consistent with their potential role in depth estimation and bilateral feature integration. Together, our analysis provides a brain-wide framework for understanding visual processing in *Drosophila*, highlighting the distinct roles of feedforward, feedback, and bilateral pathways.

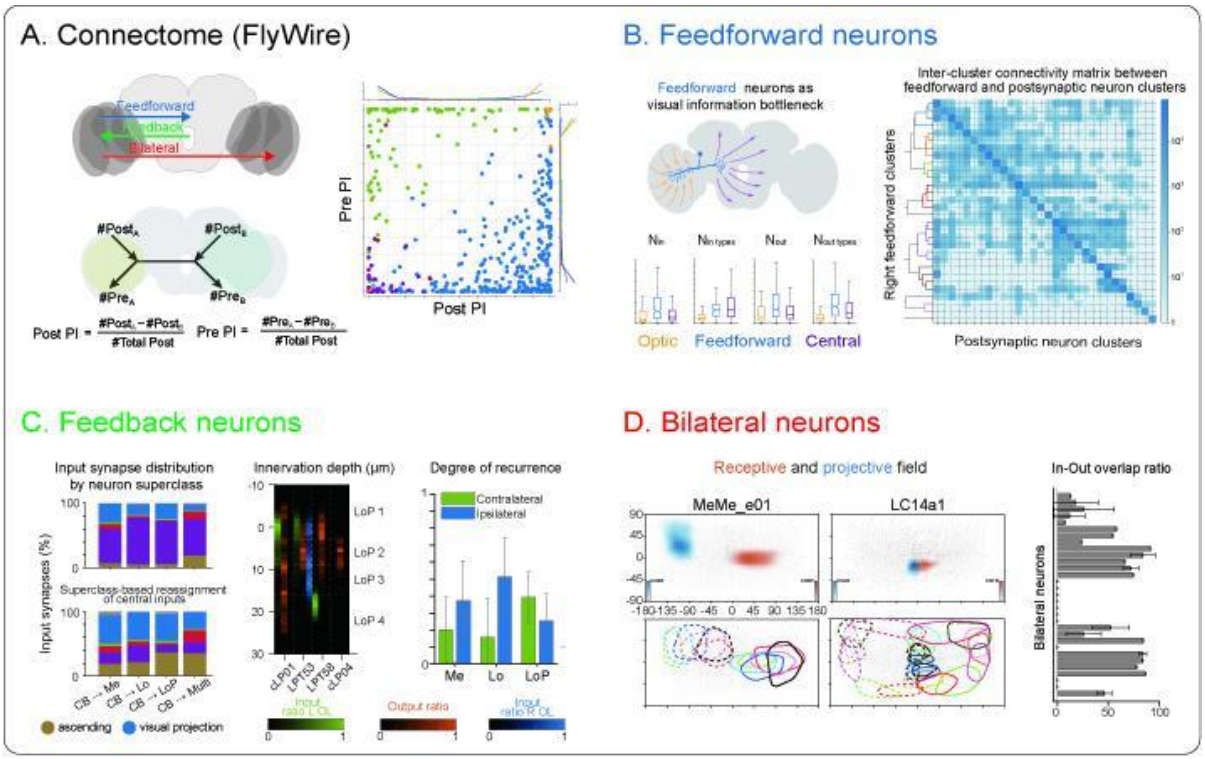

Figure 1. Feedforward, feedback, and bilateral visual pathways in *Drosophila* revealed by a whole-brain connectome.
(A) Classification based on synaptic distribution. Top left, schematic of the three neuron categories identified from the whole-brain connectome. Bottom left, Post-PI and Pre-PI, defined by the spatial distribution of pre- and post-synaptic sites, serve as classification criteria. Right, two-dimensional plot of Post-PI and Pre-PI values for neurons connecting the right optic lobe and the central brain. (B) Feedforward neurons as a visual information bottleneck. Top left, diagram illustrating feedforward neurons mediating information flow from the optic lobe to the central brain. Bottom left, counts and diversity of connected pre- and postsynaptic neurons across brain regions. Right, inter-cluster similarity matrix with dendrogram revealing modular organization based on postsynaptic targets. (C) Input characteristics and recurrent motifs of feedback neurons. Left, proportions of neuronal superclasses providing direct (top) and disynaptic (bottom) inputs. Middle, example layers of disynaptic inputs and outputs across bilateral optic lobes. Right, proportion of synapses forming recurrent motifs. (D) Receptive and projective field correspondence in bilateral neurons. Left, example heatmaps (top) and population contours (bottom) of receptive and projective fields. Right, bar graph quantifying their spatial overlap across bilateral neuron types.

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Poster

PSTR418: Connectomics II

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Title: High-throughput volume electron microscopy reveals synaptic architecture of cortico-cortical circuits in the primate prefrontal cortex

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Abstract: Over the past decade, brain connectomics using large-volume electron microscopy (vEM) has enabled detailed analysis of cortical synaptic wiring, including columnar-scale connectomes. In this study, we developed an efficient vEM imaging pipeline to investigate the synaptic microcircuitry of the prefrontal cortex (PFC) in the common marmoset, a primate species. We first modified an automated tape-collecting ultramicrotome (ATUM; RMC Boeckeler, Tucson, AZ) to achieve precise control of cutting timing, enabling stable collection of over 1,000 serial ultrathin sections (50 nm thickness) consistently aligned on slot of the Grid tape. These sections were imaged at high resolution (2.38 nm/pixel) using a high-throughput transmission electron microscope (TEM) system, Blade (Voxa, Seattle, WA), integrated with a JEM-1010 (JEOL, Tokyo, Japan). This approach allowed us to acquire ultrastructural data spanning a 1.7 × 1.2 mm cortical area in a reasonable imaging time, about a month. To examine reciprocal cortico-cortical connectivity, we co-injected anterograde (GFP) and retrograde (td-Tomato) viral tracers into Brodmann area 10 (A10), labeling projection pathways between cortical columns in areas 9 (A9) and 10 of the marmoset prefrontal cortex. Confocal laser scanning microscopy (LSM; Nikon AX, Japan) was used to image labeled axons and dendrites in A9, followed by vEM imaging of the same regions using the Blade-TEM pipeline. We revealed that anterogradely labeled axon terminals predominantly innervated dendritic spines of pyramidal neurons (89%), with less frequent targeting of dendritic shafts of non-pyramidal cells (11%), likely fast-spiking (FS) basket cells. Conversely, retrogradely labeled pyramidal neurons mainly formed synapses onto other pyramidal dendrites (65%), with some innervation of FS basket cells (32%) and rare contacts with double bouquet cells (2%).These findings provide novel insights into the synaptic organization of cortico-cortical circuits in the primate PFC and highlight the power of vEM in resolving complex neural connectivity at nanometer-scale resolution.

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Poster

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Title: Expansion-assisted light-sheet microscopy: an accessible and scalable protocol for mapping axons in whole mouse brain.

Authors: *E. IMBIMBO[1,2,3], N. BRADY[2,3], C. MANZANI[2], M. PIERONI[4], F. CASTELLI[5], M. ROFFILLI[5], B. SILVA[4,6,7], L. SILVESTRI[2];
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Abstract: Understanding the functional architecture of neuronal circuits across the entire brain requires imaging tools capable of resolving connectivity at both the macro- and microscale while preserving 3D spatial context. Current methods face critical trade-offs: *i*) physical sectioning is time consuming and disrupts long-range projections and volumetric integrity; *ii*) clearing based techniques lack the resolution necessary for single-projection mapping; *iii*) current expansion microscopy (ExM) protocols are technically demanding and time-intensive, and produces very fragile and hard-to-handle samples. To overcome these limitations, we developed an innovative imaging platform that combines the strengths of all these techniques enabling **high-resolution immunolabeled mapping of neural circuits** in intact murine brains, achieving synaptic resolution.
Our ***~4x* expansion-enhanced approach allows molecular-specific targeting** of neuronal subpopulations and biomarkers -- unattainable with endogenous fluorescence-based protocols -- without the need for physical sectioning, via a streamlined, rapid, and cost-efficient method. Combining ExM and tissue clearing based techniques with a custom-made light sheet microscope paired with a novel automated real-time 3D tracking acquisition system, this method can capture long-range projections in their native volumetric arrangement. This advancement represents a paradigm shift in multiscale brain mapping, transcending regionally confined studies, bridging whole-brain architecture with histological details to propel organism-level connectomics. This platform enables the mechanistic dissection of circuit plasticity in neurodegeneration, trauma, and neurodevelopmental disorders, embodying the axiom that for decoding the structure of the whole brain, a systematic interrogation of macro-microcircuit interactomes in intact samples is essential.

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Poster

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Human Brain Optical Mapping), by RICTD2025_2026-CUP: B97G24000240005,

Title: Exploring the 3D cyto- and myeloarchitectural organization of the human brain at multiple scales with SHORT-Ex

Authors: *D. DI MEO[1], J. RAMAZZOTTI[3], B. LORENZON[4], E. IMBIMBO[5], G. MANNUCCI[6], L. PEREGO[4], M. SORELLI[4], S. BRADLEY[2], G. MAZZAMUTO[7], D. MARTELLA[6], F. S. PAVONE[8], I. COSTANTINI[9];
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Abstract: The brain's complexity arises from the dynamic interplay of structures functioning simultaneously across multiple spatial scales. Deciphering the three-dimensional multiscale organization of neurons and their connections across large volumes of the human brain is crucial for understanding the structural basis of neural function and dysfunction. Achieving this, however, remains a significant challenge, requiring high-resolution volumetric imaging techniques such as light-sheet fluorescence microscopy (LSFM) and two-photon fluorescence microscopy (TPFM), combined with specialized optical clearing and labeling strategies. Despite these advances, the spatial resolution of these microscopy techniques is often limited to ≈ 250 nm by the diffraction of light, preventing the direct visualization of structures smaller than this threshold. In this study, we introduce *SHORT-Ex*, a specialized tissue processing and physical

expansion protocol designed to enable multiscale 3D imaging of the cytoarchitectural and myeloarchitectural organization of human brain tissue. This pipeline leverages the *SHORT* (SWITCH-H_2O_2-antigen Retrieval-TDE) method for clearing and neuronal labeling, combined with a dedicated myelinated fiber staining protocol employing the lipophilic DiD probe to obtain the 3D reconstruction of large (cm-size) human brain slabs, using either LSFM or TPFM. Regions of interest identified from these reconstructions are subsequently subjected to a further delipidation process and embedded in a swellable hydrogel, resulting in uniform and isotropic tissue-hydrogel expansion (≈ 4×), while preserving fluorescence labeling of neuronal bodies and myelinated fibers. With this approach, the diffraction limit of the imaging system is overcome, enabling super-resolution volumetric imaging with a conventional TPFM setup. As a result, nanoscale structures, such as individual myelinated axons, can be visualized and traced within volumetric human brain samples. This approach provides a powerful means for conducting comprehensive 3D structural analysis throughout the entire spatial hierarchy of the human brain, from large-scale connectivity down to single axons and subcellular compartments, enabling new insights into the fundamental principles of brain function and the structural mechanisms driving neurodegenerative disorders.

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Poster

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Title: A semi-automated pipeline for histology to MRI registration for macaque allowing surface mapping of connectivity data

Authors: *Y. HOU[1], N. VINÇON[1], T. COALSON[2], Y. HALIMI[1], T. IKEDA[5], B. HIBA[6], N. RICHARD[6], E. REID[2], M. F. GLASSER[3], T. HAYASHI[7], J. VEZOLI[1], D. C. VAN ESSEN[4], H. KENNEDY[1];
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Abstract: Understanding brain function increasingly relies on integrating data from histology, transcriptomics and multimodal imaging such as fMRI. However, aligning 2D histological sections with MRI data remains challenging, particularly in large brains such as those of macaques and humans. In this study, we developed a semi-automated pipeline for mapping retrogradely labeled neurons from histological sections onto a macaque MRI template surface. A U-Net-based machine learning algorithm was used for automated segmentation of white matter and major subcortical structures—including the claustrum, putamen, and amygdala—throughout the brain based on Nissl stain. Manual corrections were completed and used to train the model. The identified structures were contrast-enhanced on the Nissl-stained sections to facilitate subsequent 2D-to-MRI registration. For MRI data, cortical and white matter surfaces were reconstructed and aligned to a macaque MRI template using the HCP-style pipeline. The SARM atlas was registered to individual T1w image to help identify subcortical structures detected in histology. Contrast was enhanced in the cortical ribbon and subcortical regions on T1w images to improve registration accuracy. Histological sections were aligned with the corresponding MRI slices by first selecting the optimal angle that best matched the histological cutting plane, followed by linear and nonlinear 2D registration. The transformation field was used to project the labeled neuron onto the MRI slices, enabling 3D reconstruction of labeled cells. Cell density was calculated and mapped onto the cortical surface using connectome workbench.

Pipeline accuracy was evaluated by comparing variability of manual and semi-automated annotations using repeat tracer injections. Semi-automated annotations showed lower variability. Spatial alignment error, assessed by Euclidean distances between corresponding landmarks in histology and MRI, revealed a median error of 0.57 mm and a mean of 1.56 mm, well within the range of the 0.5 mm MRI resolution.

This pipeline enables high-resolution mapping of connectivity and analyzing projections at a finer scale than traditional cortical parcellations. By aligning tracer data to a common MRI template surface, it facilitates cross-study integration, flexible re-parcellation, and comparison across imaging modalities. For example, comparing the surface mapping results of tract tracing with dMRI based tractography, enables improved tractography evaluation. The pipeline can be also applied to the areal boundaries defined in histology, as well as the spatial transcriptomic data.

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Poster

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Topic: J.03. Anatomical Methods

Support: NIBIB R00 EB032217

Title: Highways of the brain: mapping dense white-matter projections at single-axon resolution with X-ray Holographic Nano-Tomography (XNH)

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Abstract: Mapping long-range brain connectivity can provide key insights into brain structure and inter-region communication, which can inform clinical research in psychiatry and neurological disease. However, widely used imaging techniques for connectivity are either limited in their ability to densely resolve individual projections (e.g. MRI and optical imaging) or prohibitively time- and resource-intensive to apply to large volumes in mammalian brains (e.g. volume EM). There is thus a need for new imaging approaches that can bridge the gap between micro- and macro-scale imaging in mammalian brains.
X-ray Holographic Nano-tomography (XNH) is a promising technique that makes use of high-energy synchrotron X-rays and phase-contrast imaging to resolve large volumes. The high penetration power and sub-nanometer wavelength of X-rays allows for the resolution of sub-100nm structures within millimeter-scale samples. At this scale, neuronal structures such as myelinated axons and large dendrites can easily be identified and traced across long distances. XNH is thus especially useful for reconstructing long-distance connections between brain regions.
Here, we demonstrate mesoscale mapping of cortical white matter connectivity within a region containing multiple sensory and association areas. We trained a convolutional neural network (CNN) to automatically segment individual axons within the XNH image data and performed proofreading to extract single-axon trajectories. We characterize organizational principles of these axon trajectories, revealing substructures within the white matter, and relate this organization to long-range cortico-cortical and cortico-subcortical communication.

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Poster

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Topic: J.03. Anatomical Methods

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Howard Hughes Medical Institute
Janelia Research Campus Graduate Research Fellowship

Title: Structure and function of an entire vertebrate brain

Authors: *A. B. CHEN[1], M. PETKOVA[1], M. JANUSZEWSKI[3], J.-M. LUECKMANN[5], A. DEV[6], G. IHRKE[7], J. W. LICHTMAN[1], V. JAIN[4], F. ENGERT[2];
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Abstract: A fundamental idea in systems neuroscience is that the functional response properties of neurons depend in large part on their synaptic connections, but testing these hypotheses has been challenging. Ideally, one would want a dataset consisting of both synaptic resolution connectivity, acquired with volumetric electron microscopy, for a circuit, as well as the activity, acquired using functional calcium or voltage imaging, of all its constituent neurons. For much of the history of neuroscience, such an experiment was technically infeasible, and it remains so in mammals, for which the largest scale functional connectomics efforts in the cerebral cortex span less than 0.5% of the brain's total volume. Here, we bridge neuronal activity and connectivity in an entire larval zebrafish brain. We leveraged the small size and optical accessibility of the larval zebrafish by first performing whole-brain calcium imaging to record the functional responses of a majority of the larval zebrafish brain's roughly 100,000 neurons during a variety of visually driven behaviors including motor learning, flash-induced startle, optomotor response, and futility-induced passivity. The animal was then fixed, stained, sectioned, and imaged via scanning electron microscopy to obtain a synaptic-resolution anatomical volume. Machine learning-based automatic segmentation methods were applied to generate a preliminary segmentation. Finally, the functional imaging dataset was registered to the electron microscopy volume to combine functional and anatomical information for each neuron. We present preliminary findings based on manual reconstruction of circuits of interest illustrating the utility of this dataset for generating hypotheses and testing existing circuit models generated from functional imaging alone.

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Poster

PSTR418: Connectomics II

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Program #/Poster #: PSTR418.13/UU15

Topic: J.03. Anatomical Methods

Support: NIH Grant U01NS132181

Title: Multimodal, multiscale imaging to build an atlas of the human brainstem

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Abstract: The human brainstem comprises ~1 billion neurons and houses complex local circuits from which large-scale projections emerge. From cell type-specific patterns of dendritic arborization to nuclear architecture, brainstem connectivity is crucial for maintaining vital functions of life and is implicated in many neuropsychiatric disorders. We are building a multiscale atlas of the human brainstem at an array of resolutions, sufficient to visualize centimeter-scale networks and cellular-level information needed to assess connectivity at micron resolution. These advances are made possible by the integration and technical advancement of a suite of imaging modalities, including magnetic resonance imaging (MRI), polarization-sensitive optical coherence tomography (PS-OCT), hierarchical phase-contrast tomography (HiP-CT), light-sheet fluorescence microscopy (LSFM), tissue clearing, immunohistochemistry (IHC), and 2-photon expansion microscopy (2PEM). We developed a 9.4T MRI protocol and applied it to an initial *ex vivo* hemi-brainstem sample, acquiring 3D structural images at 80 µm resolution with spatial distortions corrected using our Edge-Preserving Intensity Correction algorithm. We also acquired 3D single-shell diffusion MRI (dMRI) at 350 µm, revealing high-quality tractography of major pathways. For future samples, we are developing a custom radiofrequency coil to optimize acquisition, and a multi-shell dMRI protocol with higher b-values to enhance microstructural modeling and fiber orientation specificity. Following MRI, a custom 3D-axis PS-OCT rig was used to acquire images of the same sample at 6x6x3.5 µm optical resolution, providing direct measures of 3D axonal orientation. Molecular, morphological and spatial properties of cells and their projections will be assessed by applying a multi-labeling LSFM-based IHC protocol to hemi-brainstem tissue slices; DiD staining will be used to label myelinated fibers, YOYO1 for cell identification, and tyrosine hydroxylase (TH) for noradrenergic neurons and sparse labeling of axons with an isotropic resolution of 1 μm^3. Tissue will be imaged by 2PEM after 4x expansion to trace single axons and validate PS-OCT measurements, with PS-OCT serving as a geometric reference free from cutting-induced deformations. Micrometer resolution data will enable quantitative atlasing of the entire brainstem and whole-hemisphere MRI, ameliorating the limitations of *in vivo* imaging for diagnosing brainstem dysfunction. Continued development of these technologies will further illuminate

structure-function relationships beyond the brainstem, from the cellular scale to brain-wide networks.

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Poster

PSTR418: Connectomics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.14/UU16

Topic: J.03. Anatomical Methods

Support: NIH/ NINDS RF1NS023945–28
NIH/NINDS R01 23945

Title: Global corticocortical connectivity and cortical networks in rats

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Abstract: In the present study, we examined the organization of global corticocortical connections by injecting retrograde tracers, Fast Blue and Fluoro-Gold, in various cortical areas in rats (n=34). Labelled cell bodies were reconstructed in Neurolucida, then merged with our existing database and rigorously registered to a reference cortical space derived from the Paxinos atlas. For each of 39 cortical areas we quantified the density and laminar origin of projection neurons. This approach allowed us to generate a high-resolution, layer-specific connectivity matrix and to make the following qualitative observations. 1) The perirhinal (PER), entorhinal (EC), and ventral orbital (VO) areas send widespread projections to several cortical regions. Labeled cells were mainly observed in deep layers in PER and EC and in superficial layers (L2) in VO. Labeled cells were also present in upper layers in PER when a tracer was injected in higher cortical regions. 2) The VO and lateral (LO) orbitofrontal cortex have separate cortical connections. VO sends projections to several cortical regions, including retrosplenial (RSC), anterior cingulate (ACC), V1, V2M, prelimbic (PL), PER, posthrinal (POR), and EC, many of which receive no or few projections from LO. VO and interconnected cortical areas may play important roles for visuospatial functions. In contrast, LO sends more restricted projections to cortical regions such as PL, insula, PER, and EC. **3)** EC, PL, medial orbital (MO), VO, RSC,

ACC, PER, and POR receive hippocampal projections from the subiculum/CA1. **4)** RSC was one of few cortical regions receiving the strongest projections from the dorsal subiculum and presubiculum (PrS), which suggests the possibility that RSC, together with EC, may be one of the main cortical pathways between cortex and dorsal hippocampus. **5)** In sensory and motor cortical regions including somatosensory-motor, visual, and auditory cortical areas, the main connections were observed in the same sensory or motor cortical regions. Labeled cells were observed in both upper and deep layers in most labeled regions. By applying advanced statistical modelling, we seek to reconstruct the latent architecture of corticocortical connectivity—delineating subnetworks and clarifying their hierarchical relationships. In parallel, we are testing whether the hierarchical organization of cholinergic basal-to-cortical projections reported by Varsanyi et al. (2025) mirrors this corticocortical network topography.

Disclosures: L. Zaborszky: None. **H. Kondo:** None. **P. Varsanyi:** None. **Z. Nadasdy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ZN holds options in Zeto, Inc., Santa Clara, CA, and in Visie, Inc., Austin.

Poster

PSTR418: Connectomics II

Location: SDCC Halls B-H

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Topic: J.03. Anatomical Methods

Support: the National Science Foundation of China (32271430, 62361166631)
the Ministry of Science and Technology of China (2023YFF0715904)

Title: Microcircuit Architecture of Pyramidal Cell Clusters in Layer II of Mouse Medial Entorhinal Cortex (MEC)

Authors: *T. SHI[1], R. ZHANG[2,1];
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Abstract: The medial entorhinal cortex (MEC) plays a central role in the modular organization of grid cells, contributing significantly to signal amplification and neural synchronization. Within the MEC, pyramidal cell clusters display a distinctive hexagonal distribution of grid points embedded in a modular architecture. However, their internal organization and associated microcircuits remain poorly understood.We investigate the microstructural organization and circuit architecture of pyramidal cell clusters in layer II of the MEC by leveraging a large-scale electron microscopy (EM) dataset derived from 0.6 mm^3 mouse MEC tissue, obtained through continuous ultrathin sectioning and multi-beam scanning electron microscopy.To reconstruct and analyze these circuits, we employ advanced artificial intelligence techniques for neuron segmentation, verification, tracking, classification, and refinement. This enables a detailed

characterization of the local circuitry. Ultimately, our analyses and simulations aim to elucidate the microconnectivity mechanisms that underlie neural synchronization within these pyramidal clusters, providing new insights into their functional architecture.

Disclosures: T. Shi: A. Employment/Salary (full or part-time): Institute of Artificial Intelligence, Hefei Comprehensive National Science Center. **R. Zhang:** A. Employment/Salary (full or part-time): Institute of Artificial Intelligence, Hefei Comprehensive National Science Center, Fudan University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; the National Science Foundation of China (32271430, 62361166631), the Ministry of Science and Technology of China (2023YFF0715904).

Poster

PSTR418: Connectomics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.16/UU18

Topic: J.03. Anatomical Methods

Support: 1RF1MH126882-01A1

Title: All optical circuit mapping of transcriptomically defined local circuits

Authors: *W. CUNNINGHAM[1], M. ECONOMO[2], M. MOYA[2];
[2]Biomed. Engin., [1]Boston Univ., Boston, MA

Abstract: The recent surge of large-scale transcriptomic datasets has revealed the rich diversity and high specificity of cell types in neural circuits. Understanding how these cells are wired at the level of transcriptomically defined cell types is essential for linking molecular identity to circuit architecture. Current methods for mapping synaptic connectivity lack the throughput to characterize how these types connect. Here, I demonstrate a new method—Massively-parallel All-optical Paired Synaptic Electrophysiology (MAPSE)—to map local synaptic connectivity in acute slices with cell-type resolution at unprecedented scale. By combining single-cell optogenetic stimulation with high-speed voltage imaging to simultaneously detect unitary synaptic potentials across populations of cells, MAPSE enables interrogation of tens of thousands of connections in minutes. Importantly, the all-optical basis of this method allows us to readily integrate synaptic connectivity measurements with highly multiplexed fluorescence in situ hybridization, thereby unlocking the correspondence between a cell's local connectivity patterns and its specific cell type. MAPSE has the potential to significantly advance our understanding of how transcriptomically defined neuronal populations are integrated into functional circuits, providing the scalability and molecular resolution needed to construct comprehensive, cell-type-specific wiring diagrams across the brain.

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Poster

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Program #/Poster #: PSTR418.17/UU19

Topic: J.03. Anatomical Methods

Title: Integration of protein barcodes with dense morphological neuronal reconstructions

Authors: H. G. J. DAMSTRA[1], J. M. MICHALSKA[1], S. PARK[2,1], A. SHERIDAN[1], J. LYUDCHIK[1], S. CHAN[1], M. WU[1], A. MEERAN[1], C. WANG[1], J. CHEN[1], J. LEE[1], J. AXUP[1], C. MAGNO[1], S. TRUCKENBRODT[1], ***K. G. C. LEEPER**[1], J. WINNUBST[1], A. C. PAYNE[1]; [1]E11 Bio, Alameda, CA; [2]The Francis Crick Inst., Alameda, CA

Abstract: Optical connectomics has the potential to revolutionize neuroscience by resolving connections between individual neurons at scale. Expansion microscopy has recently gained recognition as a method for resolving cellular and tissue ultrastructure through visualization of protein density, including for optical connectomics. However, single-channel morphology data suffers from high human proofreading demands and are inherently unsuited to bridging spatial gaps, e.g. between non-continuous tissue sections. Recently, we introduced PRISM, a scalable pipeline that relies on cell-filling protein barcodes and custom machine learning pipelines to accurately reconstruct sparsely labeled neurons. Here, we demonstrate the integration of sparse protein barcodes, high resolution molecular annotations, and dense morphological labeling to generate data suitable to reduce or eliminate proofreading bottlenecks and gap crossing errors.

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Poster

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Topic: J.03. Anatomical Methods

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NWO-AES; P21-30, project 4

Title: Bib-mstem method for large scale connectomics

Authors: *M. KORMACHEVA[1], D. BRAULT[1], A. KIEVITS[1], M. NIESSEN[2], S. KARAÇOBAN[3], T. PALS[2], C. BOSCH PIÑOL[4], S. KHAN[4], A. T. SCHAEFER[4], J. HOOGENBOOM[3], A. A. WANNER[1];
[1]Paul Scherrer Inst., Villigen, Switzerland; [2]Delmic, Delft, Netherlands; [3]Delft Univ. of Technol., Delft, Netherlands; [4]Francis Crick Inst., London, United Kingdom

Abstract: Volume electron microscopy is the only technique to date that provides both sufficient resolution (<20 nm) and sufficient field of view (>100 µm) for the dense reconstruction of the connectome - a neuronal wiring diagram. Two currently leading systems - multi-beam SEM [1, 2] and gridtape-based automated TEM [3, 4] - achieve this by relying on serial ultrathin sectioning. However, serial collection of ultrathin sections is delicate and inherently prone to failures and artefacts contributing to over 50% of the errors in automated segmentation and requiring tens of hours of manual proofreading per neuron.To address these challenges, methods involving the collection of thicker sections (100-10000nm), and iterative sub-milling using gas cluster (GCIB) [5] or broad ion beam (BIB) [6] to match the desired resolution in the Z-axis, were developed. We introduced BIB-mSTEM - a method combining BIB for iterative sub-milling and multi-beam STEM [7] (FAST-EM Delmic, Netherlands). As a proof of concept, we collected 150 serial 250nm thick sections of rOTO-stained tissue and subsequently imaged them using the BIB-mSTEM method, producing a volume of 200×200×37.5 µm³. We now further optimise the collection method to reduce information loss due to debris, as well as imaging parameters and the reconstruction algorithm to decrease information loss between consecutive sections. BIB-mSTEM thus provides a scalable and robust alternative to traditional ultrathin serial section EM, reducing the artefacts that negatively affect the connectomics reconstruction.
References:

[1]AL Eberle et al., Journal of Microscopy 259 (2015) p. 114-120. https://doi.org/10.1111/jmi.12224; [2]P Kruit and Y Ren, Microscopy and Microanalysis 22 no. S3 (2016) p. 574-575. https://doi.org/10.1017/S143192761600372; [3]X Yin et al., Nature Communications 11, 4949 (2020). https://doi.org/10.1038/s41467-020-18659-3; [4] J S Phelps et al., Cell 184 issue 3 (2021). https://doi.org/10.1016/j.cell.2020.12.013; [5] K J Hayworth et al., Nature Methods 17 (2020) p. 68-71. https://doi.org/10.1038/s41592-019-0641-2; [6] T Templier, eLife 8 e45696 (2019). https://doi.org/10.7554/eLife.45696; [7] J Fermie et al., Microscopy and Microanalysis 27.S1 (2021) p. 558-650. https://doi.org/10.1017/S1431927621002439

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Poster

PSTR418: Connectomics II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.19/UU21

Topic: J.03. Anatomical Methods

Support: NIH Grant U01NS137250-01

Title: Neurd 2.0: end-to-end machine learning-based automated proofreading and feature extraction

Authors: *B. CELII[1], I. WESTERN[1], M. HU[4], R. LAW[5], D. XENES[2], H. MARTINEZ[3], M. ROBINETTE[1], C. SMITH[6], C. A. BISHOP[3], B. A. WESTER[2], P. K. RIVLIN[7], J. REIMER[4]; [2]Res. and Exploratory Develop., [3]Res. and Exploratory Develop. Dept., [1]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [4]Neurosci., [6]Dept of Neurosci., [5]Baylor Col. of Med., Houston, TX; [7]Res. and Exploratory Develop., Johns Hopkins Applied Physics Lab., Laurel, MD

Abstract: In the current era where electron microscopy (EM) datasets can reach millimeter scale at nanometer resolution (Shapson-Coe et al., 2021; Consortium et al., 2021), automatic segmentations can produce highly detailed 3D reconstructions of individual cells (3-D meshes). However, these reconstructions still exhibit merge and split errors and thus require laborious post-hoc proofreading in order to analyze these volumes. Although some machine learning and heuristic approaches to post-segmentation error correction exist, no current solution satisfactorily fixes all error types across multiple datasets. To begin to address this need, we present an iteration to NEURD, a heuristic-based error correction package that we have successfully applied to correct a variety of error types in the MICrONS and H01 datasets (Celii et al., 2025). NEURD decomposes each meshed neuron into a compact and extensively-annotated graph representation using manually tuned parameters, and then applies a set of hard-coded graph filters to resolve merge errors (achieving synaptic precision scores of > 87% for both axon and dendritic segments on both the MICrONS and H01 datasets). NEURD 2.0 implements this decomposition and graph classification process as an end-to-end machine learning solution, so complex merge and split error resolutions can be learned from data without laborious manual parameter tuning. Specifically, the new model employs an iterative graph contraction pipeline with sparse encoding mechanisms to perform the decomposition and extract interpretable features, and then uses a graph neural network classification head for merge error detection. This new network can easily learn and replicate the results of the original NEURD model when trained on the synthetic hard-coded graph filter merge error predictions (accuracy = 0.98, precision = 0.92, recall = 0.89, total nodes N = 165151, error nodes N = 16086). Furthermore, when trained as a binary node classifier, the unique graph filter error types can be recovered through unsupervised clustering of internal node activations in the GNN (Ex: Gaussian mixture model with 10 components over 12916 error nodes achieves 0.765 homogeneity), which demonstrates the potential for this model to discover unique error classes on future unlabeled datasets. With split error resolution then framed as a task of predicting the extension candidate least likely to be filtered away by our merge error detection model, our NEURD 2.0 model represents an advance towards a generalizable and comprehensive automated proofreading tool for EM datasets.

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Poster

PSTR418: Connectomics II

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Topic: J.03. Anatomical Methods

Support: NIH Grant UG3MH126864
NIH Grant UM1NS132207
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Title: Characterizing structural diversity of human white matter at single-axon resolution

Authors: *W.-Q. YU[1], E. E. TURSCHAK[1], K. TAKASAKI[1], S. J. COOK[2], R. TORRES[1], O. GLIKO[1], C. LAUGHLAND[1], E. PERLMAN[1], K. VILLALOBOS[1], E. GUADARRAMA[1], A. HELLEVIK[1], S. CHATTERJEE[1], R. REID[2];
[2]Neural Coding, [1]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Understanding the structural organization of the mammalian brain at the scale of individual axons is crucial, particularly for white matter, which constitutes a significant portion of the human brain. However, resolving and tracing individual axons over long distances in human white matter has historically been challenging. To address this, we have developed a method—axonal connectomics—that integrates advanced histological techniques with high-throughput light-sheet microscopy. By employing tissue clearing and expansion microscopy, we achieve dense axonal labeling in thick sections of mammalian tissue, including human brain, and image them at high resolution using a custom-built light-sheet microscope: expansion-assisted selective plane illumination microscopy (ExA-SPIM). We also built a scalable computational pipeline that uses U-Net-based segmentation to identify and skeletonize individual axons. These skeletons are aligned across serial sections to reconstruct large 3D volumes, enabling local statistical analysis even before manual proofreading. Applying this pipeline to several 1-cm^3 white matter samples from a neuropathologically normal adult brain revealed striking diversity in axonal organization. Some areas contained axons traveling in many directions without forming discrete bundles, while others exhibited well-defined fascicles with interwoven trajectories. Even in regions with one or two dominant orientations, axons could be either bundled or dispersed. Multiple structural patterns were often present within a single cubic centimeter, with some transitions between patterns occurring abruptly. To quantify these structures, we performed both image-based and skeleton-based analyses across scales to derive local and global axon orientation distributions. In line with our observations, some regions lacked a single dominant fiber orientation, instead exhibiting a more uniform arrangement. Even in regions with a predominant orientation, many axons traveled in non-aligned directions, demonstrating significant local variability. These findings highlight that white matter organization underlying the cortex is diverse and intricate, characterized often by interwoven axons. The developed pipeline enables systematic characterization of white matter axon organization at single-axon resolution across different brain areas. The long-term goal is to use this capability to trace individual axons from their cortical origin through the white matter to their destinations, providing a new understanding of large-scale brain connectivity.

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Poster

PSTR418: Connectomics II

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Topic: J.03. Anatomical Methods

Support: NIH Grant UG3MH126864
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Title: Building an interoperable, scalable pipeline to yield neuronal resolution axon traces from peta-voxel scale, whole-brain, light-sheet microscopy data

Authors: *C. LAUGHLAND[1], R. TORRES[1], K. TAKASAKI[2];
[1]Allen Inst., Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The generation of large-scale multi-dimensional image data is challenging enough, compounded by the even more rate-limiting step of data processing. Without a flexible and robust infrastructure to manage storage, resource allocation, and computational speed, processing and analyzing raw data at pace with its generation is unlikely. This challenge motivates our scalable design, offering local and distributed parallelism, variable data compression, and the leveraging of cloud resources. Moreover, the pipeline is designed to facilitate interaction at all steps, enabling feedback of results to histology and microscopy experts in a process of end-to-end pipeline optimization.
Our pipeline begins with light-sheet imaging of tissue sections using an inverted selective plane illumination microscope (iSPIM), with data continuously streamed to a network storage server. Here, image tiles are deskewed and converted from raw acquisition files to a next-generation file format (OME-zarr) that includes a deep multi-resolution image pyramid. Following data conversion, a deep learning-based U-Net model segments axons from 3D image stacks, using training data derived from fast marching of manually proofread skeletons. Segmentation outputs are post-processed through thresholding, connected component analysis, and skeletonization to produce short axonal fragments. These fragments are scored and merged using a classifier trained on geometric features, yielding longer, biologically plausible reconstructions. Tiles are then stitched into full sections via cross-correlation alignment, and skeletons are merged across tile boundaries. Adjacent sections are co-registered using coarse image alignment followed by iterative endpoint matching of skeletons to compute affine and nonlinear transforms. The final output is a compressed, portable set of skeletons representing long-range axonal projections.
By integrating machine learning-based segmentation, efficient skeletonization, and scalable registration techniques, our approach enables automated tracing of axons across physically

sectioned whole-brain volumes, facilitating high-throughput connectomics reconstruction at unprecedented resolution and scale.

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Poster

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Topic: J.03. Anatomical Methods

Support: NIH Grant UG3MH126864
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NIH Grant GR046850

Title: Mapping axonal organization and endo-lysosomal burden in Alzheimer's disease via post-mortem tissue and hiPSC models

Authors: *E. E. TURSCHAK[1,2], K. TAKASAKI[1], S. J. COOK[1], W.-Q. YU[1], K. VILLALOBOS[1], E. GUADARRAMA[1], R. TORRES[1], C. LAUGHLAND[1], O. GLIKO[1], A. HELLEVIK[1], S. CHATTERJEE[1], J. E. YOUNG[2], R. REID[1];
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Abstract: Axonal organization in the human brain remains incompletely characterized, particularly in the context of neurodegenerative diseases like Alzheimer's disease (AD). This presents a critical gap, as accumulating evidence suggests that abnormalities in axonal structure (and specifically the emergence of dystrophic axons) occur early in AD and may be linked to disruptions in endo-lysosomal (ELN) trafficking. However, due to a tradeoff between optical resolution and spatial extent in volumetric imaging, axons have historically been difficult to study in human brain tissue at scale.
This project leverages an optimized tissue-clearing and expansion microscopy pipeline to enable high-throughput, single-axon resolution mapping of post-mortem human brain samples from AD and control donors. These methods overcome long-standing technical barriers in human axonal mapping, and applying these tools in the context of AD enables an unprecedented level of detail in quantifying axonal abnormalities across brain regions. We aim to characterize regional patterns of axonal dystrophy and ELN burden across disease stages, and to examine whether these features are influenced by endo-lysosomal polygenic risk score (ePRS), a genetic metric indexing susceptibility to ELN trafficking dysfunction. Complementary studies in donor-matched human-induced pluripotent stem cell (hiPSC)-derived neurons will enable mechanistic insight into how ELN burden may drive axonal pathology. By bridging post-mortem tissue

analysis with genetic risk and stem cell modeling, this project offers a new framework for understanding early axonal changes in AD and their potential as therapeutic targets.

Disclosures: E.E. Turschak: None. **K. Takasaki:** None. **S.J. Cook:** None. **W. Yu:** None. **K. Villalobos:** None. **E. Guadarrama:** None. **R. Torres:** None. **C. Laughland:** None. **O. Gliko:** None. **A. Hellevik:** None. **S. Chatterjee:** None. **J.E. Young:** None. **R. Reid:** None.

Poster

PSTR418: Connectomics II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR418.23/VV3

Topic: I.06. Social Cognition

Support: FA9550-19-1-0280
FA9550-23-1-0024

Title: Challenging conscious processing with the fly brain

Authors: *A. SIVARAJAN[1], J. XU[2], D. GROVER[3];
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Abstract: *Challenging conscious processing with the fly brain*
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Theories of conscious processing and its neural basis have thus far focused primarily on mammalian and human systems. However, amongst the invertebrates such as the fruit fly Drosophila melanogaster, there is increasing evidence for behavioral, neurophysiological, and molecular signatures indicative of attention,reward signaling, and working memory, all reported as prerequisites of conscious processing in mammals. Here, we draw a critical parallel between the neural architecture of the fly brain and that stipulated by the Global Neuronal Workspace (GNW) theory. By examining fly connectomics, we propose that a structural motif of convergence and hierarchical integration may exist in the central complex(CX) of the fly brainresembling the long-range (cross-regional), top-down organization in the GNW. Furthermore, we present results demonstrating the extent to which the connectomic complexity of the fly brain might support the organizational principles of the GNW.We investigate the roles of sustained neural activity and inhibition to link distant stimuli through connectome-based modeling under a trace conditioning paradigm. Experimental results have shown that persistent activity over an extended trace interval arises with trace learning in a disinhibitory subnetwork of the ellipsoid body(EB), a necessary precursor of conscious processing. We reproduce this result in silico using a connectome-constrained recurrent spiking neural network and show that the

connectomic neural architecture supports such processing. Second, we investigate the broadcast of this sustained information across disparate neural structures through long-range connections to local processors in a top-down fashion,resembling broadcast and ignition-like dynamics of the GNW. We, again,show that the broader neural anatomical architecture of the fly brain possibly supports functional and mechanistic features postulated by the GNW theory through connectome-based modeling. By leveraging connectomics and biology-constrained modeling, our study highlights the potential deep evolutionary roots of fundamental principles underlying conscious processing and underscore the value of connectome-informed modeling in uncovering shared motifs of cognitive neural architecture

Disclosures: A. Sivarajan: None. **J. Xu:** None. **D. Grover:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.01/VV4

Topic: J.04. Physiological Methods

Support: CIRCA Excellence Grant 2024
Parkinson Canada Grant 2025

Title: Development of surface-enhanced Raman spectroscopy optophysiology to study neurotransmission

Authors: *S. PAQUEREAU--GABOREAU[1], A. GEDDIS[2], S. BASHIR[2], J.-F. MASSON[2], L.-E. TRUDEAU[3];
[1]Neurosciences, Univ. of Montreal, Montréal, QC, Canada; [2]Chem., [3]Pharmacol. and Physiol., Univ. of Montreal, Montreal, QC, Canada

Abstract: Neurotransmitters are essential chemical messengers that ensure neuronal communication and that regulate key brain functions, including memory, mood and movement. Disruptions in this communication can lead to serious disorders and are present in most neurodegenerative diseases including Parkinson's. Although traditional techniques such as electrophysiology and electrochemical sensing have contributed greatly to understand these alterations, they are often limited to the detection of a single neurotransmitter at a time, or lack sufficient temporal resolution when analyzing multiple neurotransmitters. To overcome these difficulties, we developed surface-enhanced Raman spectroscopy (SERS) optophysiology, an innovative approach which combines SERS with optogenetics in a fluid biological environment using glass electrodes covered with gold or silver nanoparticles. SERS enables precise molecular detection by amplifying Raman signals, while optogenetics enables targeted control of neuronal activity. This combination enables the simultaneous, real-time detection of several key neurotransmitters, such as dopamine, glutamate, GABA and acetylcholine, during neuronal activation. This approach builds on previous work by Lussier et al. (2017), who demonstrated

that SERS nanosensors can dynamically detect dopamine in the vicinity of live neurons in primary cultures, with sub-second resolution and high molecular specificity. During development, we optimized the use of this method in various neuronal cultures, where we successfully detected the expected neurotransmitters, reflecting typical neurochemical profiles. We then applied this technique on the surface of acute mouse brain slices and observed clear neuronal activity, both during optogenetic stimulation and under basal conditions, demonstrating the potential of this approach for in situ, real-time monitoring of complex neurochemical dynamics. These results were cross-validated with fast-scan cyclic voltammetry (FSCV), which confirmed the reliability of the signals detected. Once fully validated, this technique will be applied to mouse models of Parkinson's disease to study early neurochemical alterations and identify new therapeutical targets. This interdisciplinary project combines neuroscience, chemistry and engineering to push back the boundaries of real-time analysis of brain neurotransmitters dynamics.

Disclosures: S. Paquereau--Gaboreau: None. **A. Geddis:** None. **S. Bashir:** None. **J. Masson:** None. **L. Trudeau:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.02/VV5

Topic: J.04. Physiological Methods

Support: NSF Grant 2411567
NSF Grant 2143140
AFOSR Grant FA9550-21-1-0117

Title: Fabrication of multisite neural probe for dopamine mapping

Authors: *V. S. BAPANAPALLI, C. S. LIPSCOMB, K. A. WHITE, M. DARROUDI, B. S. JOHN, B. N. KIM;
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Abstract: The human brain consists of complex neuronal networks that interact electrically and neurochemically on both spatial and temporal scales. Dopamine (DA) is the most commonly found catecholamine in the brain. DA is instrumental in cognitive processes and behaviors driven by reward. Therefore, there is a critical demand for novel neurotechnologies capable of accurately mapping the spatial and temporal distribution of neurochemicals, such as DA, to better understand anomalous pathways such as those caused by neurodegenerative disorders. Existing technologies, such as carbon-fiber electrodes and neurochemical fMRI, suffer from low spatial and/or temporal resolution. In this work, we have developed a multisite neural probe that is capable of monitoring the activity of neural spikes and DA. The probe integrates 64 neuroelectrodes (32 neurochemical electrodes and 32 electrophysiology electrodes, Fig. 1a-b).

The probes were fabricated using contact lithography and plasma etching; resulting in a probe that is capable of simultaneous recording of 32 neurochemical electrodes in vivo. The electrodes and the connecting traces are made from gold, and the traces are insulated with silicon dioxide (Fig. 1a). The gold electrode is tested by performing fast-scan cyclic voltammetry (100 V/s scan rate, -0.4 - 0.7 V, 10 Hz frequency) which showed high DA sensitivity (135.4 nA/µM, Fig. 1c-d). With the high spatiotemporal resolution, the novel multisite neural probe can be used to identify the distinct role of heterogeneous distribution of DA, both spatially and temporally, in neuromodulations including directing motor control, motivation, reward, and cognitive function.

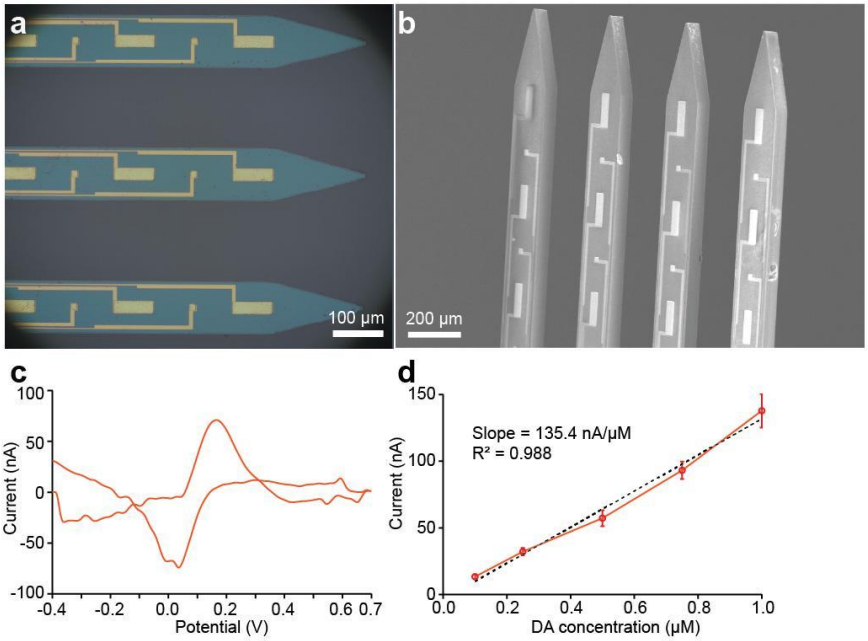

Figure 1. Optical (a) and SEM (b) images showing electrode openings and sidewalls. Background subtracted CV curve (c) shows the redox current signature of DA. The sensitivity curve (d) shows the neurochemical electrodes' capability in detecting DA.

Disclosures: V.S. Bapanapalli: None. **C.S. Lipscomb:** None. **K.A. White:** None. **M. Darroudi:** None. **B.S. John:** None. **B.N. Kim:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.03/VV6

Topic: J.04. Physiological Methods

Support: KAKENHI 23H05450

Title: Localized laser carbonization of parylene-C for seamless integration of carbon electrodes in neural interfaces

Authors: *V. G. CASTILLO, Y. SUNAGA, K. TSO, Y. OHTA, J. OHTA;
Inst. for Res. Initiatives, Nara Inst. of Sci. and Technol., Ikoma City, Japan

Abstract: Integrating high-performance carbon electrodes into complex neural interfaces remains a significant challenge. While carbon-based electrodes are known for their exceptional electrochemical stability, biocompatibility, and electrical conductivity, the high temperature processing needed is often incompatible with soft and flexible substrates or electronic chips required for next-generation neural devices.
In this work, we present a novel neural interface fabricated through localized laser carbonization of parylene-C, a widely used insulating polymer in neural device manufacturing. This laser carbonization approach enables direct integration of carbon electrodes onto any surface coated with parylene-C. Using this technique, we seamlessly integrated carbon electrodes onto implantable CMOS-based image sensors, creating an implantable multimodal brain activity monitoring platform. Using this device, we achieved simultaneous *in vivo* recordings of calcium fluorescence signals and extracellular electrophysiological activity. Following electrical stimulation of CA3, we observed a decrease in overall fluorescence in the CA1 region, which occurred alongside a reduction in local field potential power across multiple frequency bands. Interestingly, we also identified a subregion where fluorescence increased, potentially reflecting the increases observed in delta band.
Extending the capabilities of this multimodal device to a multi-electrode configuration on a single chip is a key next step. Thus, we systematically investigated the effects of laser carbonization parameters and electrode dimensions. We found optimal and effective carbonization at approximately 200 µW laser power for 11 minutes, confirmed by the distinct D and G peaks in the Raman spectrum. Across various electrode sizes (20, 50, and 100 µm), the carbon electrodes consistently exhibited significantly lower impedance compared to bare gold electrodes. Finally, we recorded *in vivo* extracellular electrophysiological signals using these different-sized carbon electrodes to evaluate how electrode size affects recording quality.

Disclosures: V.G. Castillo: None. **Y. Sunaga:** None. **K. Tso:** None. **Y. Ohta:** None. **J. Ohta:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR419.04/Web Only

Topic: J.04. Physiological Methods

Support: NIH Training Grant T32NS115667
NIH Award No. R21EB030140

NSF Grant No. 2319139
NSF Grant No. 2309323

Title: A Thermoresponsive Semiconducting Ionic Biogel for Multimodal Neurohaptic Interfaces

Authors: *A. DUTTA[1], M. DAS[1], L. MENG[1], W. ZHANG[1], H. CHENG[2];
[1]Pennsylvania State Univ., State College, PA; [2]Engin. Sci. and Mechanics, Penn State Univ., University Park, PA

Abstract: Advancing neurohaptics requires bioelectronic interfaces capable of both high-fidelity neural recording and precise somatosensory stimulation, particularly over hair-covered scalp regions. Conventional electrodes are limited by poor conformality, high impedance, and inadequate signal resolution. To address these challenges, there is a critical need for thermoresponsive, semiconducting materials that can enable in situ signal amplification through organic electrochemical transistors (OECTs). Such materials offer the potential to amplify EEG signals directly at the scalp interface, enhancing the spatiotemporal precision of neural decoding during haptic stimulation. Here, we present an ionic biogel inspired by membraneless organelles, designed to simultaneously achieve thermoreversibility, bioadhesion, semiconductivity, and electrochemical stability for long-term, through-hair neural interfacing. This composite integrates a phase-separated PEDOT:PSS-EMIM:TFSI ionic network with a gelatin-glycerol-salt hydrogel matrix, forming a metastable, liquid-liquid phase separation system that permits rapid sol-gel transitions near physiological temperatures (~40°C). The resulting material demonstrates a very low scalp impedance (~1.5 kΩ at 4 kHz), high strain-insensitivity, and exceptional charge injection capacity (10 mC/cm² at 33 Hz), enabling both robust EEG acquisition and transcutaneous electrical nerve stimulation (TENS). Furthermore, the material serves as a semiconducting channel in a tip-shaped vertical OECT, which adheres to the scalp even with hair, achieving a high transconductance of 44 mS at 40°C with temperature-gated, phase-reversible behavior. We show that this ionic biogel enables detection of event-related desynchronization (ERD) in the alpha and beta EEG bands during mechanical and electrical stimulation, with frequency-tuned cortical responses reflecting mechanoreceptor resonance (50 Hz and 500 Hz). Notably, repeated TENS induces adaptive changes in sensory perception and EEG dynamics, revealing plasticity in somatosensory pathways. This work establishes a novel class of multimodal ionic biogels tailored for neurohaptics, providing a unified material platform for both stimulation and electrophysiological monitoring through hair-covered scalp regions.

Disclosures: A. Dutta: None. **M. Das:** None. **L. Meng:** None. **W. Zhang:** None. **H. Cheng:** None.

Poster

PSTR419: Probe Design and Engineering II

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Program #/Poster #: PSTR419.05/VV7

Topic: J.04. Physiological Methods

Support: Ministry of Science Grant 25-BR-02-02
Ministry of Science Grant 25-BR-07-01
NRF Grant 2021R1C1C2011489

Title: Thermal Strain-Induced Autonomous Cuff Electrode for Peripheral Nerve Interfaces

Authors: *S. LEE[1], J. EUN[2], N. CHOU[2];
[1]KBRI, Kyungpook national university, Daegu, Korea, Republic of; [2]KBRI, Daegu, Korea, Republic of

Abstract: Peripheral Nerve Interfaces (PNIs) are technologies that establish a direct connection between the peripheral nervous system and external devices (such as computers, robotic limbs, and stimulation devices), facilitating the exchange of information. Cuff electrodes, a common type of neural electrode for these interfaces, are typically secured by a structure that wraps around the nerve's exterior. However, contemporary cuff electrode designs often rely on suturing or other mechanical fixation methods, which can lead to several complications, including nerve compression, post-surgical misalignment, and foreign body reactions. These challenges impede long-term reliability and complicate their clinical translation. To overcome these limitations, we introduce a thermo-responsive, autonomous cuff electrode (Auto-Cuff) fabricated using a shape memory polymer (SMP) that activates at body temperature. The SMP structure is engineered to undergo a shape transformation near 36-37°C, transitioning from a planar configuration into a wrapped form that encircles the nerve In this study, we developed a thermally responsive cuff electrode based on a shape memory polymer (SMP) optimized for physiological conditions. By adjusting the ratio of base monomers and crosslinking agents, the SMP was engineered to exhibit a transition in mechanical properties near body temperature, allowing for flexible, conformal contact with the nerve surface while minimizing mechanical irritation. The fabrication process incorporated a bilayer structure to precisely control thermomechanical deformation and ensure structural stability, and mechanical evaluations including tensile resistance and adhesion strength were performed.In vivo experiments using a rat sciatic nerve model demonstrated that the device could be implanted without the need for sutures or adhesives, while maintaining stable positioning and consistent electrophysiological signal acquisition over an extended period. Immunohistochemical and imaging analyses confirmed minimal inflammatory response and excellent tissue integration at the implantation site. These results demonstrate that SMP-based neural interfaces provide mechanically adaptive and biocompatible platforms suitable for chronic implantation, with potential applications in neuromodulation therapies, neuroprosthetics, and fundamental neuroscience research

Disclosures: S. Lee: None. **J. Eun:** None. **N. Chou:** None.

Poster

PSTR419: Probe Design and Engineering II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.06/VV8

Topic: J.04. Physiological Methods

Support: Yonsei University College of Medicine 6-2025-00400
NRF Grant RS-2021-00100871

Title: Impedance-based dopamine detection via a flexible neural probe for neurological disorder research

Authors: *D. PARK, C. JUNGSIK, S. PARK, H.-J. CHOI;
Yonsei Univ., Seoul, Korea, Republic of

Abstract: Dopamine (DA) is a critical neurotransmitter that plays a central role in regulating affective states, cognition, and motor functions. Abnormal dopamine signaling is closely associated with a range of neurodegenerative and psychiatric disorders, including Parkinson's disease, schizophrenia, and addiction. Consequently, the development of reliable and real-time dopamine sensing technologies is of great importance for both fundamental neuroscience research and clinical diagnostics. Conventional dopamine detection methods, such as fluorescence imaging and electrochemical techniques—including fast-scan cyclic voltammetry (FSCV)—have contributed significantly to our understanding of dopaminergic systems. However, these approaches often suffer from limitations such as insufficient temporal resolution, interference from background signals, and the requirement for external electrical stimulation, which may perturb the physiological environment and obscure subtle neurotransmitter dynamics. In this poster, we report an impedance-based dopamine sensing platform utilizing a flexible neural probe functionalized with dopamine-specific antibodies. The flexible architecture ensures mechanical durability and biocompatibility in vivo, while the antibody-based functionalization enables selective interaction with dopamine molecules without the need for enzymatic conversion or voltage cycling. This strategy allows for label-free, real-time monitoring of dopamine dynamics under minimally invasive conditions, with high sensitivity in the nanomolar per minute (nM/min) range. Our results demonstrate that the impedance variations induced by dopamine binding correlate linearly with dopamine concentration, and the sensor maintains high specificity even in the presence of common interfering molecules such as ascorbic acid and uric acid. Furthermore, the system eliminates the need for high-voltage biasing or rapid voltage sweeps, which are typically required in FSCV, thereby reducing tissue damage and preserving physiological fidelity. This impedance-based detection method offers a paradigm shift in neurotransmitter monitoring, combining high resolution, real-time responsiveness, and compatibility with chronic in vivo experiments. The proposed technology not only enables detailed exploration of dopamine-related signaling pathways in both physiological and pathological contexts but also lays the groundwork for future development of closed-loop neuromodulation systems and brain-machine interfaces tailored to dopaminergic activity.

Disclosures: D. Park: None. **C. jungsik:** None. **S. Park:** None. **H. Choi:** None.

Poster

PSTR419: Probe Design and Engineering II

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Program #/Poster #: PSTR419.07/VV9

Topic: J.04. Physiological Methods

Support: MSU fuondation Funding
National Science Foundation CAREER grant
Michigan State University Startup grant
National Science Foundation Graduate Research Fellowship

Title: An insect-based bioelectronic sensing system combining flexible dual-sided microelectrode array and insect olfactory circuitry for human lung cancer detection.

Authors: *X. LIU[1], S. W. SANCHEZ[2], Y. GONG[3], Z. JIANG[5], C. CONTAG[4], D. SAHA[2], W. LI[2];
[1]Michigan State Univ. Neurosci. Program, East Lansing, MI; [2]Biomed. Engin., [3]Electrical and computer engineering, [4]Michigan State Univ., East Lansing, MI; [5]Electrical and Computer Engin., Electrical and Computer Engin. Dept., East Lansing, MI

Abstract: Early detection of lung cancer significantly enhances treatment outcomes, yet current screening methods are limited by accessibility, sensitivity, and cost. This study introduces a bioelectronic sensing platform that integrates the highly sensitive locust olfactory system with a flexible dual-sided microelectrode array (MEA), for robust, noninvasive, and label-free detection of volatile lung cancer biomarkers. Using an innovative folding-annealing fabrication technique and PEDOT:PSS surface functionalization, we developed flexible, dual-sided MEAs with high electrode densities of 463, 687, and 766 channels/mm² across prototypes, maintaining low impedance (within 4×10^4 Ω). These MEAs demonstrated mechanical flexibility and stability, enabling direct insertion into locust brain tissue without mechanical reinforcement and facilitating precise recording of neural activity in the antennal lobe triggered by lung cancer-related volatile organic compounds (VOCs) from low concentration (1 ppm). Advanced dimensionality reduction techniques applied to the electrophysiological recordings identified distinct neural response patterns to each VOC biomarker and the complex "scent" emitted from various cell lines. Using high-dimensional population neuronal response analysis with a leave-one-trial-out approach, the platform achieved a 100% classification success rate for unknown VOCs. Additionally, varying concentrations (ppm-ppb) of individual VOC biomarkers were detected and classified with an accuracy of 86%. The system was further tested for its ability to detect and classify human lung cancer cell lines based on the unique "scent" of cultured cells, including two non-small cell lung cancer (NSCLC) and two small cell lung cancer (SCLC) types. Quantitative assessments demonstrated that the platform achieved a classification accuracy of 85% across these cell lines. These results substantiate the platform's potential for enhancing clinical diagnostics through the accurate identification of lung cancer stages and cell types. By integrating biological sensory systems with advanced bioelectronics, this study introduces a novel and efficient approach to lung cancer biomarker detection. It provides a non-invasive, brain-based cancer screening method, offering an accessible and innovative solution for early lung cancer diagnosis.

Disclosures: X. Liu: None. **S.W. Sanchez:** None. **Y. Gong:** None. **Z. Jiang:** None. **C. Contag:** None. **D. Saha:** None. **W. Li:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.08/VV10

Topic: J.04. Physiological Methods

Support: NINDS/BRAIN Initiative 1U01NS131406

Title: A Miniaturized, Multimodal Wireless Implant for Physiological Sensing Across the Gut-Brain Axis

Authors: *M. WU[1], C. H. GOOD[2], A. BANKS[2], N. J. SPENCER[3], J. ROGERS[1];
[1]Northwestern Univ., Evanston, IL; [2]Neurolux Inc, Northfield, IL; [3]Flinders Univ., Adelaide, Australia

Abstract: The gut-brain axis (GBA) is a bidirectional communication network that governs energy balance and homeostasis, while also modulating cognition, motivation, mood, and memory. While small animal models are essential for elucidating GBA mechanisms, current technologies lack fully implantable capabilities of simultaneous, long-term, multimodal recordings across multiple organ systems in conscious, untethered animals. This limitation hampers efforts to understand physiological dynamics that rely on complex inter-organ communication during naturalistic behavior.

We present a fully implantable, battery-free, wireless device enabling multisite, chronic neuro-physio-behavioral monitoring in freely moving mice. This miniaturized platform captures synchronized biophysical signals from the brain, stomach, intestine, skeletal muscle, and heart—spanning both central and peripheral systems. It supports multimodal sensing of electrophysiological activity (neural, muscular, cardiac), thermal signals (core body temperature), and mechanotactic parameters (respiration and heartbeat), and integrates microscale optoelectronic components for optogenetic stimulation. Wireless data transmission and cloud-based storage enable continuous, remote monitoring with minimal behavioral interference due to the system's compact, fully internal design.

We validated this platform in a mouse model of eating disorder, combining voluntary exercise with restricted feeding, with free-fed animals as controls. Throughout the progression of activity-based anorexia, the device continuously recorded brain, gut, muscle, cardiac, and thermal signals, while external systems tracked feeding behavior, wheel-running activity, water intake, and body weight. All data streams were synchronized and time-stamped, enabling precise correlation of internal physiological states with external behaviors. Our findings demonstrate that the platform supports uninterrupted, unsupervised, 24/7 monitoring and enables detailed analysis of physiological changes before and after anorexia onset.

This technology addresses a major unmet need in behavioral neuroscience research by enabling

comprehensive, longitudinal, inter-organ recording in small animals. It opens new opportunities for studying gut-brain signaling in health and disease and may be extended to other organ systems or therapeutic neuromodulation applications, offering broad utility for physiological studies through systems neuroscience approaches.

Disclosures: M. Wu: None. **C.H. Good:** None. **A. Banks:** None. **N.J. Spencer:** None. **J. Rogers:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

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Program #/Poster #: PSTR419.09/VV11

Topic: J.04. Physiological Methods

Support: NIH Grant 1RF1 NS126061
NIH Grant P30DA018310

Title: Nano-dialysis probes for ultra-localized and multiplexed in vivo monitoring of brain neurochemicals

Authors: *K. LI, W. SHI, A. G. ARMSTRONG, M. GUO, G. GUPTA, H. IYER, A. MAHAJAN, J. XIANG, J. V. SWEEDLER, Y. VLASOV;
Univ. of Illinois, Urbana-Champaign, Urbana, IL

Abstract: The study of neurochemicals in the brain has been critical for a plethora of clinical advancements in the field of neuroscience. Current micro-dialysis devices used to analyze neurochemical levels, however, offer poor spatial and temporal resolution, while often causing significant tissue damage due to their size. The Application-Specific Integrated Nano-Dialysis (ASIND) platform technology promises to achieve highly localized (90µm), sensitive (attomoles), and fast (sub-sec) sampling of multiple neurochemicals simultaneously from the alive and behaving mammalian brain[1]. The ASIND technology has been developed to yield a verified library of standard microfluidic components that enable user-specific integration of various functionalities (multiplexed chemical sampling, local drug delivery, electrophysiology, electrochemistry, optogenetic stimulation, analyte droplet segmentation, on-chip analyte storage, on-chip capillary electrophoresis, and nano-electrospray ionization and MALDI micro-printing) into a system-on-chip on a silicon implantable neural probe with significantly reduced cross-section (<1000 μm^2) equivalent to just a few neuron bodies across. We demonstrated successful ultra-localized measurements of *in-vivo* concentration gradients for samples collected in a mouse S1 cortex (n=6 animals) using our prototype ASIND 1.0 probes. The multiplexed CE-MS quantification of 5 neurotransmitters and 11 metabolites showed that while most of them are distributed uniformly across somatosensory cortex, glutamate and proline exhibit large gradients across two S1 subregions. In addition, we analyzed the extracellular neuropeptidome collected in-vivo in mouse S1 cortex (n=4 animals) with LC-MS, identifying 46 extracellular peptides that

indicate a complex extracellular peptide landscape shaped not only by local, but also by long-distance signaling[2].

Disclosures: K. Li: None. **W. Shi:** None. **A.G. Armstrong:** None. **M. Guo:** None. **G. Gupta:** None. **H. Iyer:** None. **A. Mahajan:** None. **J. Xiang:** None. **J.V. Sweedler:** None. **Y. Vlasov:** None.

Poster

PSTR419: Probe Design and Engineering II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.10/VV12

Topic: J.04. Physiological Methods

Support: NIH Grant R01NS123069

Title: Reconfigurable multifunctional neural probe

Authors: *H. HUANG[1], Y. LIU[1], E. T. GILBERT[2], D. F. ENGLISH[2], X. JIA[1];
[1]The Bradley Dept. of Electrical and Computer Engin., [2]Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Neural probes are important tools for monitoring and manipulating neurocircuitry in both neuroscience research and clinical applications. Most probes in use are silicon-based MEMS devices. They possess dense microelectrode arrays, but they are stiff and brittle. Additionally, commercially available silicon probes with multimodal capabilities are extremely expensive due to additional complexities involved in their fabrication. The thermally drawn fiber-based neural probe is an inexpensive biocompatible interface platform for multimodal neural stimulation and recording. However, the device's complex and delicate connection process limits its feature density and scalability. Our previous device, the TDOpE probe, addresses these challenges by including a thermal tapering process that enables rapid backend connection of complex multifunctional probes. However, there's still room for improvement in areas such as electrode density and additional probe functionalities. Here, we introduce the TDOpE3 probe. An evolution of TDOpE, it possesses significantly improved electrophysiology recording capabilities, while maintaining multifunctionality. Most significantly, the probe's unique design enables reconfiguration of the probe's electrode and optical stimulation site arrangements. Such a device provides researchers with an inexpensive, flexible, reconfigurable platform for tackling complex neuroscience questions.

Disclosures: H. Huang: None. **Y. Liu:** None. **E.T. Gilbert:** None. **D.F. English:** None. **X. Jia:** None.

Poster

PSTR419: Probe Design and Engineering II

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Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.11/VV13

Topic: J.04. Physiological Methods

Support: NIH Grant R01NS123069

Title: A multifunctional modular neural interface with reconfigurable and interchangeable components in awake behaving mice.

Authors: *Y. LIU[1], H. HUANG[1], E. GILBERT[2], X. JIA[1], D. F. ENGLISH[2];
[1]Bradley Dept. of Electrical and Computer Engin., [2]Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Recent technological developments in neural interfaces have significantly enhanced their capabilities such as recording site density, and multifunctionality, like optogenetics, drug delivery. However, current neural interfaces lack modularity and reconfigurability, limiting their adaptability across diverse neuroscience applications. Herein, we developed a modular neural probe that represents customizable configurations and interchangeable functionalities for tackling a wide range of neuroscience problems. Using the thermal drawing and thermal tapering processes, we fabricated a biocompatible neural probe with a miniaturized sensing tip (200 microns in diameter) and macro-scale backends for mounting various components. Within a 16-electrode platform, two inserted interchangeable modules were engineered: (1) silica waveguides for optogenetic stimulation, (2) microfluidic channels for pharmacological delivery. We designed a modular neural interface whose functionality can be tailored for specific research questions by freely swapping and combining multiple components after probe implantation into the awake behaving mice's brain. Here, we demonstrate its capability of high spatiotemporal resolution electrophysiology recording, precise multi-layer optogenetic stimulation, and depth-tunable localized microfluidic drug delivery. The interchangeability of the components in our system offers a major advantage in enabling the development of neural experimental designs. This advance facilitates longitudinal multimodal studies within single subjects, significantly enhancing our capability for comprehensive neural circuit investigation and therapeutic intervention development.

Disclosures: Y. Liu: None. **H. Huang:** None. **E. Gilbert:** None. **X. Jia:** None. **D.F. English:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.12/VV14

Topic: J.04. Physiological Methods

Support: NIH Grant R01NS123069

Title: High Electrode Count Depth Dependent Fiber-based Neural Probe

Authors: *N. YOUSEFPOUR, X. YANG, H. HUANG, Y. LIU, D. F. ENGLISH, X. JIA; Virginia Tech., Blacksburg, VA

Abstract: Over the past few decades, numerous biocompatible, multifunctional, and flexible neural interfaces have been developed, playing an increasingly critical role in both single-unit resolution neural recordings and the treatment of brain disorders such as epilepsy.However, the thermally drawn flexible neural probes are limited to recording signals only at the probe tip. This configuration poses significant challenges for accessing neural activity across multiple brain regions. Moreover, the extremely small diameter of the probe tip restricts the spatial distribution of electrodes to a confined region, thereby limiting the ability to record multi-regional electrophysiological signals. In addition, having high electrode counts for single neuron recording and LFP in different depth and regions of the brain is essential for getting a better understanding of neural dynamics.
Focused Ion Beam (FIB) milling allows for the high (sub-micron) resolution fabrication of electrical windows with tunable depth and geometry at distinct locations along the fiber shank. Using this technique, we fabricated a high electrode count, thermally drawn fiber probe capable of depth-dependent electrophysiology recording. In-vivo electrophysiology recording was then performed on awake and behaving mice models to demonstrate this probe's electrophysiology capabilities. Such a probe holds potential for advancing both basic neuroscience research and neurological disease models.

Disclosures: N. Yousefpour: None. **X. Yang:** None. **H. Huang:** None. **Y. Liu:** None. **D.F. English:** None. **X. Jia:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.13/VV15

Topic: J.06. Computation, Modeling, and Simulation

Support: Washington Research Foundation Undergraduate Fellowship 2024-2025

Title: Complete Automation of Simultaneous Multi-region Electrophysiology Recordings

Authors: *K. J. YANG[1], D. BIRMAN[3], N. A. STEINMETZ[2];
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Abstract: In vivo electrophysiology experiments face significant challenges in equipment management, efficiency, and reproducibility as neuroscience research increasingly demands brain-wide coverage using multiple probes simultaneously. Current approaches often limit to one or two probes and create substantial bottlenecks for understanding distributed neural activity across brain regions that engage during complex behaviors.We have developed Pinpoint, a comprehensive software platform that enables remote control and automation of multi-probe electrophysiology experiments (Birman et al. 2024). Our platform allows experimenters to control micromanipulators through a web application while providing interactive 3D brain visualization for planning insertions and sophisticated automation pipelines for experiment execution.A key innovation is the integration of a probe tracking system developed in collaboration with the Allen Institute (Parallax). This camera-based tracking system ensures accurate and reproducible probe positioning while detecting insertion failures before damage occurs, providing automatic targeting capabilities and real-time monitoring throughout the insertion process.During multi-probe experiments, our platform manages the complete insertion workflow, coordinating probe movements to entry coordinates, driving to target depths at safe speeds, and removal procedures. Pinpoint implements comprehensive safeguards to prevent brain tissue damage and inter-probe collisions.The efficiency gains are substantial. Traditional manual insertion requires fifteen minutes per probe, meaning eight-probe experiments typically require two hours of insertion time. Our automation platform parallelizes the process, reducing insertion time to nineteen minutes total on average (2 probes, n=16).These open-source tools enable reproducible, high-efficiency, brain-wide electrophysiology data collection. By integrating camera-based tracking to enable automatic targeting and insertion, Pinpoint establishes the foundation for scalable neuroscience research.

Disclosures: K.J. Yang: None. **D. Birman:** None. **N.A. Steinmetz:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.14/VV16

Topic: J.06. Computation, Modeling, and Simulation

Support: Mary Gates Endowment for Students

Title: Systematic quantification and correction of mouse brain atlas discrepancies for optimized in vivo electrode targeting

Authors: *R. H. YIN[1,2], K. E. MILLER[2], N. A. STEINMETZ[3];
[2]Neurobio. and Biophysics, [3]Biol. Structure, [1]Univ. of Washington, Seattle, WA

Abstract: Comprehensive and accurate brain atlases are an instrumental prerequisite for neuroscientists, allowing for navigation to identified brain regions based on anatomical location. Inaccuracies in these atlases lead to unquantified inconsistencies in brain area targeting. With the

advent of new tools for large-scale electrophysiology that target multiple regions across the brain, the requirements for accurate targeting have become even more critical. Qualitative observations of existing atlases (e.g. Paxinos and Franklin, Allen Institute, and MRI-defined spaces) have revealed inconsistencies in the delineation and alignment of anatomical structures. Here we systematically quantify and experimentally validate the accuracy of mouse brain atlases to real-world coordinates of the in vivo brain within the mouse skull. To assess the agreement among different mouse brain atlases, we evaluated sets of atlas-defined coordinates for identical stereotaxic targets. These atlas coordinates were transformed into the Common Coordinate Framework space to quantify spatial discrepancies. This analysis revealed experimentally relevant disagreement among three mouse brain atlas spaces. To experimentally validate atlas accuracy, we injected small volumes of dye to compare atlas predictions relative to in vivo targeting. Using iontophoretic injection and probe labeling with fluorescent dyes, spatial localization of dye volume in mouse brain tissue was optimized. This achieved high-contrast histochemical fluorescence detection without diffusion into untargeted brain areas, allowing for reliable measurement of true stereotaxic brain locations. We developed a computational pipeline to quantify discrepancies between in vivo injection sites and corresponding atlas-defined coordinates. To correct for these discrepancies, 3D affine transformations were applied to align each atlas space with in vivo measurements. Our results demonstrate that existing atlas spaces have non-negligible deviations from the in vivo mouse brain. We provide a quantitative basis for affine correction across mouse brain atlases, thereby improving precision of in vivo targeting. An experimentally validated atlas space is set to be employed in Pinpoint, our electrode targeting software interface, to enable the community to readily plan electrode trajectories with greater accuracy. This work contributes to the development of validated community tools and facilitates reproducible, large-scale electrophysiology.

Disclosures: R.H. Yin: None. **K.E. Miller:** None. **N.A. Steinmetz:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.15/VV17

Topic: J.06. Computation, Modeling, and Simulation

Support: Hacettepe University BAP Grant TOA-2023-20338

Title: Embedded Soft-Sensor Feedback Enables Strain-Invariant Stress Delivery in a Biaxial Cell-Stretching Platform

Authors: *S. KUMBAY YILDIZ[1], E. ÖZTÜRK[1], N. DÜZ[1], S. AKAR[3], P. DINÇER[2], I. UYANIK[1];
[1]Electrical and Electronics Engin., [2]Med. Biol., Hacettepe Univ., Ankara, Turkey; [3]Mechanical Engin., Cankaya Univ., Ankara, Turkey

Abstract: Mechanotransduction is central to numerous neurobiological processes, yet in vitro mechanostimulation studies often rely on the assumption that imposed membrane displacements directly translate into uniform and reproducible cellular stress. This oversimplification introduces variability, particularly when dealing with heterogeneous or batch-variable substrates such as PDMS. To address this critical limitation, we introduce the first biaxial cell-stretching system that employs real-time stress feedback via embedded liquid-conductive soft sensors integrated directly into the culture membrane. The sensor, based on a microfluidic serpentine channel filled with conductive liquid, tracks resistance changes at 60 Hz as a proxy for wall tension, capturing fine-grained fluctuations in mechanical loading. A custom high-speed control loop (24 kHz update rate) modulates four orthogonal stepper actuators to deliver precisely defined triangular or square waveforms (5-20% strain amplitude; 0.5-10 s period), automatically compensating for batch-to-batch PDMS elasticity differences that can span an order of magnitude. Closed-loop operation maintains stress within 2% of the target steady-state value, dramatically reducing variability across membranes of different stiffnesses (e.g., Shore 00-30 vs. Shore A-40), which would otherwise generate more than 2.5-fold differences in stress under identical strain commands. Compared to traditional open-loop strain-based control, our feedback approach reduces inter-well stress variability by over threefold. These results highlight a fundamental shift in how mechanical input can be controlled in cell culture systems—not in terms of nominal strain, but in the actual stress experienced by the cells. This distinction is crucial for reproducible mechanotransduction research. By actively normalizing the mechanical environment across substrates with different material properties, the system eliminates a long-standing confound in in vitro models. Moreover, it enables reliable comparisons across experimental conditions that were previously subject to hidden stress disparities. This stress-invariant paradigm allows researchers to confidently attribute biological outcomes—such as changes in cytoskeletal architecture or nuclear signaling—to defined mechanical inputs, rather than uncontrolled variation in material behavior. Our findings offer a transformative advance for neuroscience research seeking to dissect how cells decode mechanical cues during development, injury response, and regeneration.

Disclosures: S. Kumbay Yildiz: None. **E. Öztürk:** None. **N. düz:** None. **S. Akar:** None. **P. Dinçer:** None. **I. Uyanik:** None.

Poster

PSTR419: Probe Design and Engineering II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR419.16/VV18

Topic: J.06. Computation, Modeling, and Simulation

Support: Hacettepe University BAP Grant TOA-2023-20338

Title: A Modular Biaxial Cell-Stretching Device Reproduces Physiological Strain and Activates YAP-Dependent Mechanotransduction in Human Myoblasts

Authors: *E. ÖZTÜRK[1], N. DÜZ[1], S. AKAR[2], S. KUMBAY YILDIZ[1], P. DINÇER[1], I. UYANIK[1];
[1]Hacettepe Univ., Ankara, Turkey; [2]Cankaya Univ., Ankara, Turkey

Abstract: Accurate replication of physiological mechanical cues is essential for decoding the biomechanical regulation of neuro-muscular development, repair, and disease. We present a novel, compact, and modular biaxial cell-stretching device that combines engineering precision with biological compatibility to reproducibly deliver cyclic strain to adherent cell cultures. The device integrates a finite-element-optimized PDMS membrane design, high-torque orthogonal stepper actuators, and embedded microfluidic soft sensors to achieve spatially uniform strain across a 12 × 12 mm culture window. Programmable waveform control enables square or triangular strain profiles (5-20% amplitude; 0.1-2 Hz) with <2% steady-state error, independent of membrane stiffness variations. The design supports long-term imaging, modular component replacement, and straightforward integration with standard tissue culture protocols. Biocompatibility assays using C2C12 murine myoblasts demonstrated proliferation rates equivalent to conventional polystyrene dishes, while immortalized human myoblasts maintained >67% viability after 48 h of continuous culture on the PDMS membrane. To evaluate the platform's functional relevance, we subjected human myoblasts to 10% cyclic biaxial stretch at 0.2 Hz for 5 hours. RT-qPCR analysis revealed robust upregulation of canonical YAP/TAZ pathway genes—C-MYC (1.7×), MYL9 (4.8×), DIAPH1 (1.9×), and ANKRD1 (2.0×)—relative to static controls ($p < 0.001$), confirming the device's capacity to elicit bona fide mechanotransductive responses. These findings provide direct evidence that the device reproduces not only the mechanical characteristics of physiological strain, but also activates functionally relevant cellular signaling pathways. The specific upregulation of YAP/TAZ target genes demonstrates that the stretch delivered by the platform is interpreted by cells as biologically meaningful mechanical input—validating its use in studies of muscle plasticity, regeneration, and mechano-responsive gene expression. Importantly, the uniformity and fidelity of the delivered strain enable systematic, high-confidence comparisons between experimental conditions, including live-cell imaging of mechanotransductive events. This device thus fills a critical gap in the field by offering a reproducible, easy-to-use, and biologically validated tool for exploring how mechanical cues shape gene regulation, differentiation, and tissue architecture in neuromuscular systems.

Disclosures: E. Öztürk: None. **N. düz:** None. **S. Akar:** None. **S. Kumbay Yildiz:** None. **P. Dinçer:** None. **I. Uyanik:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.01/VV19

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R01HD070855 (Hershey)
NICHD 1R21HD112910-01

Title: Neurofilament light chain (NfL) but not glial fibrillary acidic protein (GFAP) serum levels are elevated in Wolfram syndrome

Authors: *M. JANSEN[1], H. M. LUGAR[2], C. BROWN[3], A. TANG[4], L. OIKNINE[4], L. CHEN[5], B. A. GORDON[1], F. URANO[4], B. MARSHALL[6], T. HERSHEY[2];
[2]Psychiatry, [1]Washington Univ. in St. Louis, Saint Louis, MO; [3]IM-Div. of Endocrinology, Metabolism & Lipid Res., [4]Med., [6]Pediatrics, [5]Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Wolfram syndrome is a rare autosomal recessive disorder caused by pathogenic variants in the *WFS1* gene characterized by childhood-onset insulin-dependent diabetes, optic nerve atrophy, sensorineural hearing loss, and diabetes insipidus. Neurologic symptoms and brain structural differences appear in early childhood and progressively worsen with time. There is an urgent need for efficient clinical trials targeting neurodegeneration in Wolfram syndrome. Currently established neurologic measures known to decline over time (e.g. visual acuity, thalamic and ventral pons volumes) are costly, time-consuming and require in-person assessment, specialized equipment and/or expertise to acquire. Serum neurofilament light chain (NfL) is a measure of neurodegeneration (e.g. neuro-axonal degradation) and serum glial fibrillary acidic protein (GFAP) is a measure of neuroinflammation (e.g. glial cell injury) that may provide less expensive, more accessible, standardized and repeatable alternatives to neuroimaging or behavior-based measures for clinical trials. To determine whether these measures are altered in Wolfram syndrome, serum NfL and GFAP levels were compared between participants with Wolfram syndrome (n=45) and multiple control groups, including their parents (n=55) and unaffected siblings (n=12), as well as unrelated healthy (n=29) and Type-1 diabetic (T1D) controls (n=46 NfL, 47 GFAP). Fasting blood samples were taken at the time of each research study visit, spun down and frozen at -80° C. Samples were then assayed for NfL and GFAP by Quanterix using the Simoa® Neurology 2-Plex B (N2PB) Kit. Results were validated against internal standards and statistical outliers excluded. The first available timepoint for each individual was used for this analysis. Groups were compared with general linear models controlling for age, sex, race, ethnicity, and time since sample after log10 transformation to normalize the raw values, followed by post-hoc 2 group comparisons. Groups differed on NfL ($p<.001$) but not GFAP ($p=.19$). Post-hoc comparisons revealed that NfL was higher in the Wolfram group (mean raw NfL = 12.62 pg/mL, range 3.03-44.71 pg/mL) relative to all control groups (mean raw NfL = 5.70 pg/mL, range 1.98-20.54 pg/mL). Results suggest that serum NfL but not GFAP levels are elevated in Wolfram syndrome, independent of age or diabetes status. Future analyses will examine if NfL levels change over time and whether they relate to existing measures of neurodegeneration in Wolfram syndrome. Such results will have significant implications for the design and implementation of clinical trials targeting neurodegeneration in this patient population.

Disclosures: M. Jansen: None. **H.M. Lugar:** None. **C. Brown:** None. **A. Tang:** None. **L. Oiknine:** None. **L. Chen:** None. **B.A. Gordon:** None. **F. Urano:** 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.; Received research funding from Prilenia Therapeutics, Principal investigator of an ongoing clinical trial of AMX0035 in patients with

Wolfram syndrome, supported by Amylyx Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Patent No. 9,891,231 for the use of soluble MANF in pancreatic beta cell disorders, U.S. Patent Nos. 10,441,574 and 10,695,324 for the treatment of Wolfram syndrome, beta cell death, and other endoplasmic reticulum (ER) stress-related disorders. F. Consulting Fees (e.g., advisory boards); Member of the Scientific Advisory Board of Emerald Biotherapeutics, supported by Camelot Biocapital. Other; Founder and President of CURE4WOLFRAM, INC. (2020–2025). **B. Marshall:** None. **T. Hershey:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.02/VV20

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A review of detection technique for neurodegenerative diseases (Parkinson's disease, mild cognitive impairment and Alzheimer's disease) using a magnetic finger tapping device over the last two decades.

Authors: *A. KANDORI[1], Y. SANO[1], T. MIZUGUCHI[2], S. SUZUMURA[3], T. TSUJI[4]; [1]Hitachi Ltd., Res. and Develop. Group, Next Res., Kokubunji/Tokyo, Japan; [2]Maxell, Ltd, New Business Producing Div., Yokohama, Japan, Japan; [3]Fac. of Rehabilitation, Sch. of Hlth. Sciences, Fujita Hlth. Univ., Toyoake/Aichi, Japan; [4]Grad. Sch. of Advanced Sci. and Engineering, Hiroshima Univ., Higashi-Hiroshima, Hiroshima, Japan

Abstract: Twenty years ago, we discovered that the time difference between the left and right brains in auditory-evoked magnetoencephalography was negatively correlated with MMSE scores ($p<0.001$) [1]. We thought this time difference might also affect the alternating finger tapping.
At the same time, a magnetic finger tapping device was developed to measure finger dexterity in Parkinson's disease, and its effectiveness in classifying Parkinson's disease has also been proven [2][3].
After that, we began research on Alzheimer's dementia using a magnetic finger tapping device. We found that finger tapping parameters reflect the decline in finger function associated with the advanced stages of dementia and may help assess the severity of AD [4][5]. Furthermore, we extracted 248 characteristics from the finger-tapping waveforms of 182 MCI patients and 352 normal controls, applying AI model (improved Random Forest (RF)) [6]. Our AI model achieved the highest discrimination accuracy between healthy and MCI, with an F1-score of 0.795 (recall = 0.778 and precision = 0.814).
These results demonstrate the potential of finger-tapping measurement as a highly accurate screening test for neurodegenerative diseases.
Acknolegement: We thank Dr. Hitoshi Kagaya and Dr. Izumi Kondo at NCGG for their advice

on this study.

References: [1] Oe H, et al. Predictor of cognitive deterioration in elderly subjects by auditory-evoked magnetic signal using magnetoencephalography. Neurol Clin Neurophysiol. 2004 Nov 30;2004:76. [2] Kandori A, et al. Quantitative magnetic detection of finger movements in patients with Parkinson's disease. Neurosci Res. 2004 Jun;49(2):253-60. [3] Shima K, et al. Measurement and Evaluation of Finger Tapping Movements Using Log-linearized Gaussian Mixture Networks. Sensors (Basel). 2009;9(3):2187-201. [4] Suzumura S, et al. Finger Tapping Test for Assessing the Risk of Mild Cognitive Impairment. Hong Kong J Occup Ther. 2022 Dec;35(2):137-145. [5] Suzumura S, et al. Differences in Finger Dexterity in Patients With Mild and Moderate Alzheimer's Disease-A Study of Cognitive Function by Disease Severity. Brain Behav. 2025 Mar;15(3):e70403. [6] Sano Y, et al. Detecting mild cognitive impairment by applying integrated random forest to finger tapping. Med Biol Eng Comput. 2025 Feb 1.

Disclosures: A. Kandori: None. **Y. Sano:** None. **T. Mizuguchi:** None. **S. Suzumura:** A. Employment/Salary (full or part-time): Department of Rehabilitation Medicine, National Center for Geriatrics and Gerontology. **T. Tsuji:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.03/VV21

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Novel multiplex immunoassay tools for characterizing neurological disease autoantibody profiles

Authors: G. SOLARES[1], T. AHMED[2], H. L. STEINER, III[2], S. WILLIAMS[2], L. MARQUARDT[2], D. DROLL[2], *A. J. SAPORITA[2];
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Abstract: By binding common autoantigens including synaptic proteins, neuronal antigens, cell surface receptors, and aggregation-prone proteins, autoantibodies contribute to the pathogenesis of several neurological diseases by disrupting normal neural function and promoting inflammation and tissue damage in the nervous system. To further understand neuroinflammation and support preclinical development of therapeutic strategies, we developed a novel multiplex immunoassay for 25 autoantibodies relevant to a broad spectrum of neurological disorders, including neurodegenerative diseases. Recombinant protein antigens were conjugated to magnetic microspheres and used to capture autoantibodies in biological samples such as plasma, serum, and cerebrospinal fluid (CSF). Fluorophore-coupled detection antibodies against human IgG or IgM were then incubated with the immunocomplex and plates were read on the xMAP INTELLIFLEX® DR-SE platform. Specificity was verified with biotinylated control antibodies against each target paired with a streptavidin-phycoerythrin

reporter. We profiled a cohort of samples from presumed healthy controls and individuals with Alzheimer's disease, stroke, multiple sclerosis, or Parkinson's disease using our AAW™ Automated Assay Workstation while simultaneously testing IgG and IgM autoantibodies by leveraging the dual reporter functionality of the xMAP INTELLIFLEX® DR-SE. The dual receptor assay modification replicated the results from the single-channel experiment performed by the AAW™ with an average R-squared value of 0.96 for IgG autoantibodies across the sample cohort. The IgM autoantibody profile was generally higher relative to IgG in serum and plasma, likely due to the higher avidity, broader specificity, and pentameric structure of IgM. In contrast, IgM autoantibodies were mostly absent in CSF samples, consistent with the size of pentameric IgM hindering its ability to cross the blood-brain barrier. For both IgG and IgM, the presence of autoantibodies in healthy control samples suggests that detection of autoantibodies is not necessarily indicative of disease. In the neurological disease samples, elevation of IgG or IgM autoantibodies was relatively rare, but an "outlier-effect" was quite pronounced when observed, often producing MFI values well above the healthy sample range. In summary, we utilized a novel multiplex immunoassay to characterize the autoimmune response against 25 potential autoantigens in human serum, plasma, and CSF, highlighting the value of autoantibody profiling in neurological disease.

Disclosures: G. Solares: A. Employment/Salary (full or part-time): MilliporeSigma. **T. Ahmed:** A. Employment/Salary (full or part-time): MilliporeSigma. **H.L. Steiner:** A. Employment/Salary (full or part-time): MilliporeSigma. **S. Williams:** A. Employment/Salary (full or part-time): MilliporeSigma. **L. Marquardt:** A. Employment/Salary (full or part-time): MilliporeSigma. **D. Droll:** A. Employment/Salary (full or part-time): MilliporeSigma. **A.J. Saporita:** A. Employment/Salary (full or part-time): MilliporeSigma.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.04/VV22

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Standardization of brain lesioning in real time thermal monitoring radiofrequency ablation by sEEG electrodes

Authors: *S. LIM[1], J. PARK[2], W. CHANG[3];
[1]yonsei university, Seoul, Seodaemun-gu, Korea, Republic of; [2]Yonsei university college of medicine, [3]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Background: Epilepsy is the third most common neurological disorder after stroke and dementia. Although various antiepileptic drugs have been developed, surgical resection remains the best treatment option for approximately 30% of patients with drug-resistant epilepsy. Currently, radiofrequency (RF) thermal ablation is widely used in surgical resections, and

clinical data has been accumulated, ensuring the safety and efficacy of this treatment modality. However, standardizing the relationship between thermal dose and lesion volume could further enhance both the safety and effectiveness of the procedure. In this study, we aim to enhance safety by real-time monitoring of the temperature at the lesion site In vivo and to standardize lesion size based on thermal dose. **Method**: Thermal ablation was performed using a radiofrequency device developed by *Starmed*. Ablations were conducted at temperatures of 60°C, 70°C, and 80°C for durations of 10 and 30 seconds. A PMT sEEG electrode (outer diameter: 0.8 mm, length: 2 mm) was modified for use in a monopolar setup, and ablation was performed using a two channel. Hematoxylin and eosin (H&E) staining was used to evaluate lesion size. **Result**: Lesion size increased with higher temperatures and longer ablation durations. The experimental animals remained alive throughout the procedures. H&E staining revealed a clear border line, which was used to measure lesion size. **Conclusion**: Although RF ablation is widely used, there is limited information on the lesion size formed at specific temperatures. Traditionally, lesion size has been estimated using frequency parameters, which makes accurate prediction difficult. The device used in this study enables real-time temperature monitoring, allowing for safer ablation procedures and quantification of temperature-dependent lesion changes. This technology holds promise for safer surgeries and more precise lesion formation in future clinical applications. Furthermore, it holds potential for effective treatment in patients with epilepsy.

Disclosures: S. Lim: None. **J. Park:** None. **W. Chang:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.05/WW1

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Proteomic Analysis of Aqueous Humor from Glaucoma Patients Identifies Biomarker Panels Informative of Disease-relevant Clinical Parameters

Authors: *J. M. LEVENSON[1], M. C. PIZARRO PEÑARANDA[2], Y. NAM[3], A. AKHLAQ[3], W. YELLE[1], T. V. JOHNSON, III[4];
[1]FireCyte Therapeut., Boston, MA; [2]The Johns Hopkins Wilmer Eye Inst., Baltimore, MD, ; [4]Wilmer Eye Inst., [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Glaucoma is the second leading cause of blindness. Standard of care focuses on treatments that lower intraocular pressure (IOP), with therapy escalation driven by documented progression. Despite these interventions, many treated patients experience ongoing degeneration of retinal ganglion cells and associated vision loss. There are no biomarkers that predict conversion from stable to progressive disease, nor are there any biomarkers that inform on rate of decline once disease converts to the progressive state. These tools would be transformative for

patient care and dramatically hasten clinical trials of neuroprotective therapeutics.
To address this need, we analyzed the proteome of aqueous humor (AH) and plasma from patients with either stable (n=20) or progressive (n=22) glaucoma, and compared those proteomes to patients with cataracts (n=20, the control population). Proteomic analysis was performed using the Somascan™ platform, which permitted the examination of over 7,000 protein species. Patient groups were balanced demographically and the only variable that affected analysis was the use of medications to control IOP, which was associated with a significant decrease in MAG and BMPR1A in AH.
Plasma proteomes showed no significant differences across patient groups. Principal component analysis indicated AH from progressive patients exhibited a signature of increased inflammation whereas stable patients exhibited a signature of hypoxia and stress. False discovery rate statistics identified 595 proteins that were significantly different in progressive patients relative to cataract controls, and 1,195 proteins significantly different between progressive and stable patients. There were no proteins that were significantly different in stable vs. cataract patients. Pathway enrichment analysis indicated the differences in progressive vs. cataract patients were driven by inflammation, CNS development and cellular senescence, whereas differences in the progressive vs. stable patients were driven by inflammation. Publicly available cell mapping data indicated a prominent contribution by glia in both comparisons. Machine learning was leveraged to create models predictive of progression rate, visual function and patient classification.
Our results indicate that neuroinflammation is a major component of the dysfunctional systems biology underlying glaucoma and is a key feature distinguishing progressive from stable patients. Machine learning permitted rapid identification of minimal protein sets that can be deployed as diagnostic and prognostic biomarkers to support clinicians and clinical studies.

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. **M.C. Pizarro Peñaranda:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FireCyte Therapeutics. **Y. Nam:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FireCyte Therapeutics. **A. Akhlaq:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); 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. **T.V. Johnson:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); FireCyte Therapeutics.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.06/Web Only

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH R01NS131469
NIH R01NS121040

Title: Dual-energy ct-based phantom calibration for quantative assessment of brain water content in cerebral edema

Authors: *C. SCOPICE[1], B. YANG[2], J. CUSTARD[3], J. KIM[4], H. CHOI[2];
[1]UT Hlth. Houston, Houston, TX; [2]Neurosurg., UT Hlth. at Houston, Houston, TX; [3]UTHealth Sci. Ctr. McGovern Med. Sch., Houston, TX; [4]Neurosurg., UT Hlth. at Houton, Houston, TX

Abstract: ***Introduction*** Cerebral edema (CE) following subarachnoid hemorrhage (SAH) contributes significantly to early brain injury and poor neurological outcomes. Prior studies have relied on surrogate markers such as sulcal effacement, but direct and quantitative imaging of CE remains limited. Dual-energy CT (DECT) offers a promising tool for assessing brain water content (BWC), a hallmark of CE. ***Methods*** We evaluated DECT's ability to differentiate tissue compositions using brain-mimicking phantoms. Eight phantoms with varying biochemical compositions (water, glycerol, and barium sulfate) were scanned at 80 kV and 150 kV. Linear regression models assessed associations between HU values and each component. Random forest classifiers were trained using HU from 80 kV alone, 150 kV alone, and both combined. Ten-fold cross-validation was used to evaluate classification performance, and Wilcoxon signed-rank tests compared the models. ***Results*** Distinct HU distributions were found across phantoms with varying biochemical compositions. HU values at 80 kV exhibited high sensitivity to barium sulfate content ($R^2 = 0.939$, $p < 0.001$), with a strong linear relationship indicating that 1% increase in $BaSO_4$ was associated with ~197 HU increase. In contrast, 80 kV HU values were weakly associated with water ($R^2 = 0.158$, $p = 0.329$) and glycerol content ($R^2 = 0.126$, $p = 0.389$). At Sn150 kV, no statistically significant associations were observed between HU values and any individual biochemical parameter. Water content ($R^2 = 0.206$, $p = 0.259$), glycerol content ($R^2 = 0.237$, $p = 0.222$), and barium content ($R^2 = 0.188$, $p = 0.284$) all showed weak explanatory power. Random forest classifier model using 80 kV alone achieved a mean cross-validated accuracy of 79.3%, outperforming models trained on Sn150 kV alone (69.4%). When HU values from both energy levels were combined, classification accuracy increased significantly to 92.7%. Wilcoxon signed-rank tests confirmed the superiority of the dual-energy model over both single-energy models ($p = 0.00195$ for all comparisons). ***Conclusion*** These findings suggest DECT's potential for tissue differentiation and quantitative imaging in neuroimaging applications.

Disclosures: C. Scopice: None. **B. Yang:** None. **J. Custard:** None. **J. Kim:** None. **H. Choi:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.07/WW2

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Decoding cellular architecture in neurodegeneration and brain tumors using spatial proteomics: an end-to-end solution with Akoya Biosciences' PhenoCode Human Neurobiology Panel, PhenoCycler-Fusion Platform, and Visiopharm's Phenoplex Spatial Biology analysis.

Authors: D. WINKOWSKI[1], Y. PEYMANFAR[2], M. POULIN[2], R. MIHANI[2], T. BAIRD[1], *K. J. HALES[1];
[1]Visiopharm, Broomfield, CO; [2]Akoya Biosci., Marlborough, MA

Abstract: Understanding the spatial proteomics architecture of the brain is essential for understanding the cellular and molecular mechanisms that drive neurodegeneration and tumor progression. In this study, we present an integrated, end-to-end spatial proteomics workflow designed to provide deep insights into brain pathology through ultrahigh-plex imaging and advanced spatial analysis. Human brain tissues—including control samples as well as those from individuals with, Alzheimer's disease, and glioblastoma—were profiled using Akoya Biosciences' PhenoCode™ Human Neurobiology Panel on the PhenoCycler®-Fusion 2.0 platform. This high-throughput system enables whole-slide imaging at subcellular resolution while preserving fragile brain morphology through its isothermal and scalable molecular barcoding chemistry. Advanced spatial analysis was performed using Visiopharm's Phenoplex™ workflow, which offers AI-assisted cell segmentation, deep phenotyping, and neighborhood-level spatial quantification. The Human Neurobiology Panel, comprising 42 well-defined biomarkers, provides detailed cellular characterization to identify and distinguish neuronal subtypes, glial cells, and vascular components. The panel enabled identification of disease-associated cellular states, shifts in immune infiltration, and microenvironmental remodeling unique to each condition-insights that are difficult to achieve with lower-plex or bulk analysis methods and that are unique to the Phenoplex workflow. We anticipate this end-to-end spatial proteomics solution—combining Akoya Biosciences' PhenoCycler®-Fusion 2.0 system and PhenoCode™ panel with Visiopharm's Phenoplex™ analysis—will empower researchers to resolve cellular heterogeneity, map disease-specific microenvironments, and accelerate discoveries in neurodegenerative disease and brain oncology.

Disclosures: D. Winkowski: A. Employment/Salary (full or part-time): Visiopharm. **Y. Peymanfar:** A. Employment/Salary (full or part-time): Akoya Biosciences. **M. Poulin:** A. Employment/Salary (full or part-time): Akoya Biosciences. **R. Mihani:** A. Employment/Salary (full or part-time): Akoya Biosciences. **T. Baird:** A. Employment/Salary (full or part-time): Visiopharm. **K.J. Hales:** A. Employment/Salary (full or part-time): Visiopharm A/S.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.08/WW3

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Spatial multiomic profiling of Alzheimer's and Parkinson's disease mouse models using Spatomics CFP™ technology

Authors: J. CASEY[1], S. RAVVIN[1], T. RAHMAN[2], A. CHOLEWINSKI[1], R. ZHENG[3], X. WANG[3], H. WU[4], J. YOO[5], A. ACHARYA[5], P. SAVICKAS[1], ***R. CLARY**[1];
[1]HistoWiz, New York, NY; [2]HistoWiz, HistoWiz, NY; [3]Spatomics, Guilford, CT; [4]Abclonal, Woburn, MA; [5]Mol. Instruments, Los Angeles, CA

Abstract: Alzheimer's disease (AD) and Parkinson's disease (PD) are progressive neurodegenerative disorders marked by complex pathology and poor clinical outcomes. Understanding the molecular mechanisms driving these diseases is critical for therapeutic development. Advances in spatial biology now allow for nuanced resolution of molecular signatures of AD and PD.
Here, we utilized Spatomics CFPTM cleavable fluorophore technology to investigate spatial multiomic signatures in transgenic mouse models of AD and PD. The PD model (B6-hSNCA-A53T), aged 12 weeks, carries the A53T mutation in the human alpha-synuclein gene, resulting in the overexpression of phosphorylated alpha-synuclein, a marker of Lewy bodies in the brain. The AD model (FAD4T), aged 6-8 weeks, carries mutations in the genes encoding human amyloid precursor protein (APP) and human Presenilin-1 (PSEN1), confirmed by increased beta-amyloid deposition in the brain relative to age-matched controls.
Using CFP fluorophores, we tested a 9-plex protein marker panel comprising GFAP, NeuN, TMEM, Olig2, SMMHC, MBP, AIF1/IBA, CD45, and Calretinin, in conjunction with Molecular Instruments' HCRTM HiFi Probes targeting APP and alpha-synuclein RNA. The Spatomics™ technology uses cyclic immunofluorescence where antibodies are applied in sets of 4, followed by an HRP-conjugated secondary, and proprietary Spatomics™ fluorophores. After the last antibody is stripped, the tissue is imaged, and the fluorophores are cleaved before the cycle is repeated. Notably, the Spatomics™ method allows for plexing beyond 20 markers while preserving tissue integrity.
Ongoing work will apply the panel to AD and PD transgenic mouse models, in addition to healthy, age-matched control brain tissue. Visiopharm's AI-driven image analysis will quantify marker expression, co-expression, and spatial proximity to APP and alpha-synuclein transcripts. Spatial relationships to glial (GFAP, IBA1, and Olig2), neuronal (NeuN), and immune (CD45) cell markers will be mapped to identify neuroinflammatory and neurodegenerative microenvironments.
Overall, the Spatomics CFP™ platform is a powerful approach to spatially resolve the complex multiomic signatures of AD and PD to enhance our understanding of neurodegenerative disease biology.

Disclosures: J. Casey: A. Employment/Salary (full or part-time): HistoWiz. **S. Ravvin:** A. Employment/Salary (full or part-time): HistoWiz. **T. Rahman:** None. **A. Cholewinski:** A. Employment/Salary (full or part-time): HistoWiz. **R. Zheng:** A. Employment/Salary (full or part-time): Spatomics. **X. Wang:** A. Employment/Salary (full or part-time): Spatomics. **H. Wu:** A. Employment/Salary (full or part-time): Abclonal. **J. Yoo:** A. Employment/Salary (full or part-time): Molecular Instruments. **A. Acharya:** A. Employment/Salary (full or part-time): Molecular Instruments. **P. Savickas:** A. Employment/Salary (full or part-time): HistoWiz. **R. Clary:** A. Employment/Salary (full or part-time): HistoWiz.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.09/WW4

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Sino Danish Center (SDC)

Title: Monitoring brain status using cell specific extracellular vesicles

Authors: *C. VANGSØE[1], B. MUMM[2], A. STENSBALLE[3], K. R. DRASBEK[4];
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Abstract: Stroke is a devastating disease and the second leading cause of death worldwide. Acute ischemic stroke (AIS) accounts for 85% of cases, while intracerebral hemorrhage (ICH) constitutes the remainder. Currently, only treatments of AIS are clinically approved and need to be initiated within 4.5-6h of symptom onset, while recently it was shown that acute blood pressure lowering was beneficial for ICH. However, treatment strategies for the two types of strokes are detrimental for the other, thus uncovering diagnostic biomarkers is of utmost importance in correct treatment selection. Extracellular vesicles (EVs) have emerged as promising biomarker candidates and tools for diagnostics. EV subpopulations that originate from specific cell types reflect the state and biomolecular signature of their parent cells and thus have significant potential in disease and brain monitoring. In this study, we developed and validated a protocol for isolating EV subpopulations from human plasma based on their cell origin. Using magnetic bead-based immunocapture targeting cell type surface markers, we enriched specific EV subtypes from plasma. To assess disease specific fingerprints of EVs, we have been studying protein expression in several different EV subpopulations using proteomic analysis. We have found that each EV subpopulation depicted their own cell specific fingerprints. Enrichment of both EV-associated markers (e.g. CD9) and cell-specific markers (e.g. CD31 and CD56) confirmed the specificity of the isolation approach. Nanoparticle tracking analysis showed that EV subpopulations exhibited distinct particle size distributions, supporting their differential cellular origin. These findings demonstrate the ability to selectively isolate EV based on cell type from human plasma. This methodological foundation enables future investigations into stroke-specific EVs from relevant cell populations, with the potential to aid in differential stroke diagnosis.

Disclosures: C. Vangsøe: None. **B. Mumm:** None. **A. Stensballe:** None. **K.R. Drasbek:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.10/WW5

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant R21NS121589

Title: Distinct Immune Cell Signatures and Intercellular Signaling in Moyamoya Disease

Authors: *Z. DEMIRAG[1], H. UCHINO[4], K. TOKAIRIN[2], S. P. RAO[5], T. C. CHIANG[6], G. MORTON[3], A. G. LEE[5], M. Y. CHENG[6], G. K. STEINBERG[7];
[1]Neurosurg., Stanford Univ. Sch. of Med., Palo Alto, CA; [2]Neurosurg., Stanford Univ. Sch. of Med., Stanford, CA; [3]Stanford Univ. Sch. of Med., Palo Alto, CA; [4]Neurosurg., Stanford Univ., Palo Alto, CA; [5]Stanford Univ., Palo Alto, CA; [6]Neurosurg., [7]Stanford Univ., Stanford, CA

Abstract: Background: Moyamoya Disease (MMD) is a rare cerebrovascular disease characterized by the narrowing of the intracranial arteries and the formation of compensatory collateral vessels. Previous research suggests a role of elevated immune response and abnormal extracellular matrix-related gene expression in the progression of the disease. This study investigated the immune landscape and cell type-specific gene expression of peripheral blood mononuclear cells from MMD patients using single cell RNAseq (scRNA-seq) and flow cytometry. Methods: Whole blood samples were collected from two healthy controls (HC), three bilateral MMD patients, and two non-MMD patients. PBMCs were isolated within two hours after collection and processed for 10x single-cell RNA sequencing. Immune cell heterogeneity and differentially expressed genes (DEGs) in each cell type were identified. Top biological pathways were determined using Ingenuity Pathway Analysis (IPA). 11-fluorochrome flow cytometry panel was used to validate key immune populations. Specific immune populations were isolated using Fluorescence-activated cell sorting (FACS) for validation of key genes using qPCR. Results: Our results showed a distinct immune cell landscape in MMD patients, including CD8+ NKT cells, CD4+ NKT cells, and CD8+ T cell populations. DEG analyses revealed that CD8+ NKT cells, naive CD4+ T cells, and classical monocytes are more transcriptionally active in MMD. Pathway analysis shows predicted changes at the immune-vascular interface, as well as inhibition in mTOR, interleukin, and CXCR4 signaling. Multi-color flow cytometry with additional patient samples showed a significant increase in circulating CD8+NKT cells in MMD. Notably, ligand-receptor interaction analysis showed that CD8+NKT cells are major targets of intercellular communication, receiving signals from multiple immune cell types. Among these, the CD94/NKG2A receptor is a prominent inhibitory signal in this interaction. Conclusions: This study highlights MMD-specific immune cell types, cell type-specific transcriptomics and intercellular signaling networks, suggesting involvement of immune-vascular crosstalk in MMD. Ongoing studies utilize FACS isolation of CD8+NKT cells and qPCR to validate candidate genes. These results demonstrate a potential role of peripheral immune dysfunction in MMD vascular pathology.

Disclosures: Z. Demirag: None. **H. Uchino:** None. **K. Tokairin:** None. **S.P. Rao:** None. **T.C. Chiang:** None. **G. Morton:** None. **A.G. Lee:** None. **M.Y. Cheng:** None. **G.K. Steinberg:** None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.11/WW6

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

Support: NIH Grant AG068990

Title: Multimodal imaging and inflammatory correlates of neuropsychiatric symptoms across the Alzheimer's disease spectrum

Authors: *H. OH;
Brown Univ., Providence, RI

Abstract: Background: Although genetic factors associated with the aggregation of misfolded proteins clearly play a role in Alzheimer's disease (AD), an individual's susceptibility to stress, often expressed as neuropsychiatric symptoms, has been shown to confer an increased risk for AD. The goal of this study was to probe a neuro-mechanistic pathway that underlies the relationship between neuropsychiatric symptoms and AD neuropathology using multimodal neuroimaging and peripheral inflammatory markers.Method: We evaluated whether the relationship between neuropsychiatric symptoms and AD neuropathology are differentially mediated by white matter microstructural integrity, neuronal activity, and inflammatory markers along the spectrum of AD from the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset that consists of 398 cognitively normal older adults (CNs), 817 participants with mild cognitive impairment and 307 AD patients. White matter integrity and fMRI data were processed to quantify diffusion measures and neuronal activity levels. Plasma-based inflammatory markers were summarized using principal component analyses. Neuropsychiatric Inventory Questionnaire (NPI-Q) scores represented individuals' stress responses and neuropsychiatric symptoms.Results: NPI-Q scores were significantly associated with brain Aβ indices across the entire cohort. Relationships between the neural activity, stress responses, and Aβ deposition, however, differ by diagnostic groups. As stress indicators increased, CN subjects exhibited increased activity across brain regions, while AD participants exhibited decreased activity. Moreover, as brain Aβ load increased, CN participants experienced hyperactivity, while AD participants experienced hypoactivity. White matter integrity and peripheral inflammatory markers showed diagnostic group differences, but did not significantly mediate the relationship between neuropsychiatric symptoms and AD pathologies.Conclusion: These results indicate a potential link between heightened stress responses and increased susceptibility to the

development of AD pathologies through neuronal hyperactivity, especially in the early stages of AD.

Disclosures: H. Oh: None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.12/WW7

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NRF Grant RS-2023-NR077202

Title: Engineering Peptide-Imprinted Nanocomposites (EPIN) for Target Specific Extracellular Vesicle Isolation and Analysis in Neurological disorders

Authors: *J. KIM;
Asan medical center, Col. of Medicine, Ulsan Univ., Seoul, Korea, Republic of

Abstract: Neurological disorders require early detection, yet traditional diagnostic methods lack specificity and sensitivity. Blood-derived extracellular vesicles (EVs) provide a promising, non-invasive alternative for biomarker discovery in neurological disorders, despite challenges in cell-specific isolating them from brain related EVs due to their complexity and scarcity. In this study, we introduce Engineering Peptide-Imprinted Nanocomposites (EPIN) as an innovative platform for isolating cell-specific EVs from biological matrices, overcoming the limitations of conventional methods. Utilizing magnetic nanoparticles, we developed EPIN with peptide template that bind specifically to EVs surface proteins, enhancing the capture efficiency. Targeting proteins CD63, CD9, and CD81, EPIN showed high specificity in isolating EVs, compared with UC, TEI, and SEC assays. In particular, EPIN-glutamate aspartate transporter (GLAST) effectively isolated astrocyte-derived EVs in blood, indicating significant promise for monitoring neuromyelitis optica spectrum disorder (NMOSD), where astrocytopathy is a key feature. We demonstrated the utility of EPIN-GLAST using blood samples by integrating brain-derived proteins and miRNAs, establishing a platform for disease-specific monitoring. This approach extends beyond detecting astrocytic damage in NMOSD, representing a significant advancement in EV-based diagnostics. Our findings underscore the capability of EPIN-GLAST in elucidating disease dynamics and emphasize the value of targeted EVs analysis in neurological disorder research, paving the way for novel biomarker discovery.

Disclosures: J. Kim: None.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.13/WW8

Topic: J.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of a Luminex xMAP® Assay for the Pharmacokinetic Detection of the anti-MYCN PNA oligonucleotide BGA002 in MYCN-expressing tumors

Authors: *J. M. DRAGICH[1], I. BUTTARAZZI[2], S. BORTOLOTTI[3], L. CERISOLI[3], S. ANGELONI[1], L. MONTEMURRO[4], D. BARTOLUCCI[3], R. TONELLI[5,3];
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Abstract: Biogenera SpA is a biopharmaceutical company focused on the development of anti-gene therapeutics. Its leading anti-cancer compound, BGA002, is a peptide nucleic acid (PNA) oligonucleotide that targets the *MYCN* oncogene. It has received Orphan Drug designation from both the EMA and the US FDA regulatory agencies for the treatment of neuroblastoma (by EMA and FDA), soft tissue sarcomas (by EMA), and small cell lung cancer (by FDA) which are entering clinical trials. This study aimed to determine whether analytical measurements of BGA002 for clinical trial pharmacokinetics could be effectively transferred from a conventional ELISA-based format to Luminex xMAP® Technology, in support of a more efficient bioanalytical workflow. To achieve this, a custom bead-based assay was developed using xMAP Technology. Multiple probe designs were tested, including 3' amine-modified oligonucleotide capture probes and 5' biotin-labeled detection probes. Assay performance was assessed in human plasma and serum matrices, various buffer formulations, and on two different xMAP instrument platforms (the MAGPIX® System and the FLEXMAP 3D® System). The optimized assay demonstrated robust performance across matrices and platforms, with the selected conditions offering the broadest dynamic range for BGA002 detection on the MAGPIX System. xMAP Technology reduced sample volume requirements and streamlined the experimental workflow, providing clear advantages over conventional ELISA methods. These findings support the successful adaptation of Luminex xMAP Technology for the detection in pharmacokinetic studies of PNA-based oligonucleotide therapeutics such as BGA002. This platform also provides a versatile and scalable solution for bioanalytical applications in proteomics, genomics, and emerging nucleic acid-based therapies.

Disclosures: J.M. Dragich: A. Employment/Salary (full or part-time): Luminex Corporation. **I. Buttarazzi:** None. **S. Bortolotti:** A. Employment/Salary (full or part-time): Biogenera SpA. **L. Cerisoli:** A. Employment/Salary (full or part-time): Biogenera SpA. **S. Angeloni:** A. Employment/Salary (full or part-time): Luminex Corporation. **L. Montemurro:** None. **D. Bartolucci:** A. Employment/Salary (full or part-time): Biogenera SpA. **R. Tonelli:** A. Employment/Salary (full or part-time): Biogenera SpA.

Poster

PSTR420: Biotechniques and Spatial Omics: Neurodegenerative Diseases (AD, PD, MS, Stroke)

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR420.14/WW9

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

Title: Benchmarking MSD assays for quantifying exon 1 mutant Huntingtin following pharmacological inhibition of UBE2K

Authors: K. R. NICKERSON[1], S. R. WESSEL[1], K. GROVER[1], J. LAWRENCE[1], D. B. OLIVER-JOLICOEUR[1], D. CHIMMANAMADA[2], V. K. VISHNUDAS[1], N. R. NARAIN[1], *S. GESTA[1], K. M. ROSEN[1], O. ZAVIDIJ[1], M. A. KIEBISH[1];
[1]BPGbio, Waltham, MA; [2]Coorg Biosci., Arlington, MA

Abstract: The accumulation of the aggregation-prone exon 1 huntingtin (Htt) protein with expanded polyglutamine (polyQ) repeats is a key feature of Huntington's disease (HD). Accurate quantification of both soluble and aggregated polyQ-expanded Htt isoforms is crucial for assessing therapeutic strategies targeting aberrant protein processing and clearance. Meso Scale Discovery (MSD)-based assays offer high-throughput, quantitative detection of these species and are well characterized in HD mouse models (Reindl 2019, Landles 2020, Smith 2022), though validation in cell lines with overexpression of polyQ-expanded Htt has been limited. UBE2K, an E2 ubiquitin conjugating enzyme, has been previously identified as a putative therapeutic target in HD, capable of enhancing autophagy and lysosomal pathways that facilitate the degradation of mutant Htt aggregates. Here we benchmarked the established MSD assays to quantify the impact of a novel UBE2K-targeting tool compound on both soluble and aggregated polyQ-expanded exon 1 Htt in SH-SY5Y human neuroblastoma cells ectopically expressing an Htt fragment with 73 polyQ repeats. MSD measuring soluble exon 1 and aggregated Htt (Landles 2020) were used to track changes in Htt levels in response to compound treatment. In addition, Torin 1, Bafilomycin A1, or Epoxomicin were used to modify Htt homeostasis and serve as positive control. AGERA (agarose gel electrophoresis for resolving aggregates) was utilized to quantify high molecular aggregates. Significant reduction of > 50% of soluble exon 1 Htt was observed in cells treated for 24h with Torin 1, Bafilomycin A1, or Epoxomicin indicating that both autophagy and proteasomal pathways contribute to soluble Htt degradation. These effects were robustly detected using MSD. Notably, Bafilomycin A1 treatment resulted in a modest (20%) reduction of aggregated Htt as measured by MSD (n=4, p=0.023), although this was not reflected by AGERA. UBE2K-targeting for 48h **r**educed the amount of soluble exon 1 Htt by 40%. Tracking alterations in aggregated Htt by MSD and AGERA revealed no changes after 48h of treatment. Preliminary data confirms that 72h of treatment with tool compound, decrease aggregated Htt protein assessed by two orthogonal assays, MSD and AGERA. This study demonstrates that the UBE2K-targeting tool compound can potentially reduce soluble exon 1 polyQ-expanded Htt as well as high-molecular aggregates in human cellular model of HD, supporting continued investigation of its potential as a therapeutic target. MSD proved effective

for detecting changes in soluble exon 1 Htt protein while AGERA may provide more sensitive detection of changes in high molecular aggregates.

Disclosures: K.R. Nickerson: A. Employment/Salary (full or part-time): BPGbio. **S.R. Wessel:** A. Employment/Salary (full or part-time): BPGbio. **K. Grover:** A. Employment/Salary (full or part-time): BPGbio. **J. Lawrence:** A. Employment/Salary (full or part-time): BPGbio. **D.B. Oliver-Jolicoeur:** A. Employment/Salary (full or part-time): BPGbio. **D. Chimmanamada:** A. Employment/Salary (full or part-time): BPGbio. **V.K. Vishnudas:** A. Employment/Salary (full or part-time): BPGbio. **N.R. Narain:** A. Employment/Salary (full or part-time): BPGbio. **S. Gesta:** A. Employment/Salary (full or part-time): BPGbio. **K.M. Rosen:** A. Employment/Salary (full or part-time): BPGbio. **O. Zavidij:** A. Employment/Salary (full or part-time): BPGbio. **M.A. Kiebish:** A. Employment/Salary (full or part-time): BPGbio.

Poster

PSTR421: Deep or Recurrent Networks: Theory and Modeling II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.01/WW10

Topic: J.06. Computation, Modeling, and Simulation

Title: The Impact of Heterogeneity on Neural Network Learning and Memory: Insights from an in silico Model

Authors: *Y. G. TIRAT-GEFEN;
Maxwave Res. LLC, Rockville, MD

Abstract: Motivation: Biological neural networks, such as those in the human brain, are composed of diverse cell types and synaptic structures, yet exhibit remarkable regularity and organization. A fundamental question in neuroscience is how neural heterogeneity, i.e. variability in structural and neuron parameters, affects network capabilities in learning and memory. This study explores the role of such heterogeneity in shaping the performance of large-scale neural networks. Methods: We constructed a in silico model of a large neural network incorporating probabilistic distributions (e.g. specifying distribution types, mean and standard deviation) of key network parameters (e.g. number of synapses per neuron and time-delays). Machine learning techniques were used to evaluate the network's learning and memory capabilities across different parameter distributions. In parallel, we employed genetic algorithms to evolve network architectures, enabling adaptive optimization of both parameter heterogeneity and structural organization. Results: Networks with low parameter variability exhibited limited performance, while those with moderate heterogeneity achieved better learning and memory retention. However, excessive variability impaired function, indicating the existence of an optimal heterogeneity range. Furthermore, networks organized into modular clusters of internally homogeneous sub-networks outperformed globally heterogeneous or randomly structured networks, suggesting that structured heterogeneity confers functional advantages in neural networks.

Disclosures: Y.G. Tirat-Gefen: None.

Poster

PSTR421: Deep or Recurrent Networks: Theory and Modeling II

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR421.02/WW11

Topic: J.06. Computation, Modeling, and Simulation

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Title: Benchmarking different methods for neural dimensionality estimation

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Abstract: Dimensionality reduction techniques are powerful tools for obtaining information on latent neural dynamics and brain computations. The complexity of nonlinear algorithms often limits their widespread use, and linear techniques such as PCA (Principal Component Analysis) and its derivatives remain extremely popular due to their simplicity and interpretability. However, deciding how many latent components to consider is crucial, yet this is still not supported by robust consensus in the field literature. To address this issue, we benchmarked several methods for selecting the number of latent dynamics to retain when applying PCA. Our selection of criteria is aimed at providing a focused comparison among the most widely used in the neuroscience literature, such as explained variance thresholds and participation ratio (PR; Gao et al., 2017, *bioRxiv*), as well as cross-validation (CV). We also considered the most promising methods from other fields, such as the Kaiser rule (Kaiser, 1960) and parallel analysis (PA; Horn, 1965). Simulated data were at the core of our work, providing a ground truth that enables a direct comparison of the different retention criteria. Specifically, we designed a simulation procedure based on combinations of latent variables (correlated signals across units) and uncorrelated noise addition. Many parameters were varied to simulate different scenarios (e.g. number of synthetic units, noise amount etc.). To consider the different aspects of future real-world applications, we computed several performance metrics, including dimensionality error, reconstruction accuracy (i.e. the ability to recover the original, noise-free matrix) and estimated noise error (i.e. the ability to accurately estimate the total amount of noise). In short, PA and CV were found to be the most effective methods. Indeed, they produced the lowest error rates in estimating both dimensionality and noise levels, as well as achieving the most accurate reconstruction of correlated activity. These findings remained consistent across a wide range of

noise levels, population sizes, and time series lengths. In particular, neither of the methods was significantly affected by the noise regime: they did not incorporate more components as the data became noisier, unlike the other criteria. In conclusion, despite explained variance thresholds and PR being among the most widely used criteria in neuroscience, their results are extremely sensitive to noise and other factors, such as population size. Of the criteria tested, PA and cross-validation are the most promising tools for further applications to real-world neural data.

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Program #/Poster #: PSTR421.03/WW12

Topic: J.06. Computation, Modeling, and Simulation

Title: Curvature of neural manifolds reveals a tradeoff in encoding strategy

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Abstract: The discovery of low-dimensional manifold structure in neural data has given key insight into how the brain can encode and decipher information from large populations of neurons, but the geometry of these neural manifolds, and how this relates to neural computation, is not fully understood. These neural manifolds have been shown to exhibit non-trivial (that is, non-Euclidean) geometry. This is clearly demonstrated by the fact that principal component analysis (PCA), a dimensionality reduction method that projects data into lower-dimensional Euclidean space, dramatically overestimates the intrinsic dimension of data derived from common neural population models.Investigations into the shape of these neural manifolds have revealed links between geometry and encoding strategy. In fact, there are several studies finding neural manifolds that agree with the dimension and topology of the known stimulus space of the experiment. A few notable examples are ring manifolds encoding angle of head direction in head direction systems of rodents, flies, and zebrafish, and toroidal manifolds representing two dimensional spatial location. Although understanding the topology of a neural manifold can be very informative of the encoding strategy of the population, it does not fully capture the shape of the manifold in population space.While most prior work has focused on the dimension and topology of neural manifolds, this study examines extrinsic curvature as a key geometric descriptor. Extrinsic curvature quantifies the deviation of a manifold from flat, Euclidean space, and is a signature of the particular embedding of the neural manifold in population space. Curvature is calculated for a family of common information encoding strategies as a function of key individual neuron properties and population size. In a few standard decoding models, curvature is shown to capture a tradeoff between decodability and noise tolerance, and between information compressibility and specificity. Understanding the curvature of an encoding strategy

helps to answer the question: among infinitely many possibilities, why would a given neural population choose its particular representation of the stimulus?

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Title: Reliable neural manifold decoding using low-distortion alignment of tangent spaces

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Abstract: Since the advent of large-scale extracellular recordings in neuroscience and the widespread adoption of neural population frameworks, there is an increased need to achieve efficient decoding of task-relevant behavioral information from neural dynamics. The most prevalent methods to uncover neural manifolds from population dynamics are linear dimensionality reduction methods such as principal component analysis (PCA). These techniques, however, do not necessarily provide the most informative means to decode behavioral metrics. On the other hand, nonlinear manifold learning techniques enable direct access to manifold geodesics, which may improve behavioral metric decoding or provide new analysis perspectives. However, many manifold learning techniques unfortunately produce highly distorted embeddings, which severely curtails their effectiveness for neuroscience applications. Optimizing these techniques for neural data presents its own challenges, as neural data are often noisy, sparse, and non-homogeneous.
To address this, we present a bottom-up manifold learning technique, Riemannian Alignment of Tangent Spaces (RATS), which efficiently constructs and aligns low-distortion local views to obtain a global embedding. We further introduce global distortion, a measure to evaluate embeddings with respect to the geometry of the observed data. We validate our method on idealized datasets, several electrophysiological datasets, and recurrent neural network (RNN) models that recapitulate neural population activity. We embed task-based manifolds, some with

known dynamics, intrinsic dimensions, noise, sparsity, and density, and demonstrate that RATS yields the lowest distortion embeddings when compared to several manifold learning techniques. RATS can tear manifolds to recover known topologies in intrinsic dimension in recordings from head direction cells, grid cells and place cells. RATS also demonstrates improved decoding of behavioral metrics from noisy task-relevant manifolds underlying navigation, cycling, and sensorimotor tasks. Overall, our results indicate that embeddings with low distortion better decipher the elusive links between neural population dynamics and behavior, an issue that remains central to systems neuroscience.

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Program #/Poster #: PSTR421.05/WW14

Topic: J.06. Computation, Modeling, and Simulation

Title: Geometry of the temporal embeddings for neuronal spike times

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Abstract: Neural activity is fundamentally a dynamical process, yet much of neuroscience still relies on static measures such as average firing rates to decode it. These rate-based approaches neglect the temporal structure inherent in spike trains and the nonlinear dynamics underpinning neuronal computation. Inspired by Takens' theorem—a foundational result in nonlinear dynamical systems theory—we apply time-delayed embedding techniques to inter-spike interval (ISI) sequences, enabling the reconstruction of latent dynamical trajectories from single-neuron and population spiking activities. While previous work has shown that individual neuron ISI embeddings can reveal intrinsic dynamical structure of single neuron activity, here we extend this framework to multi-neuron ensembles to investigate how collective spike timing could work as a possible population code relates to behavior. As a proof of concept, we analyzed experimental data from trained mice performing sensorimotor tasks. We demonstrate that different neurons within the same circuit exhibit diverse, attractor dynamics. Importantly, these reconstructed trajectories not only capture internal dynamical motifs but also predict behaviors with high fidelity. Moreover, we identify transitions in the geometry of these embeddings that correlate with behavioral state changes, suggesting a link between dynamical phase shifts in neural activity and shifts in cognitive or motor states. Our results introduce a principled alternative to spike rate-based analyses, offering a dynamical systems approach to uncover how timing-based neural codes in both single cells and populations map onto behavior. This

framework opens new directions for understanding neural computation by bridging single-neuron dynamics, ensemble coordination, and behavior through a common geometrical lens.

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Title: Critical brain dynamics across scales: in vivo evidence for the coexistence of neuronal avalanches and the edge of chaos

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Abstract: The critical brain hypothesis posits that neural activity in the brain operates near a critical point at the boundary between order and disorder. Over the decades since this hypothesis was proposed, numerous theoretical frameworks and experimental evidence for the hypothesis have been reported. Among these, two central concepts are neuronal avalanches and the edge of chaos. Neuronal avalanches refer to a critical phenomenon related to the synchronized firing of neurons. The edge of chaos describes a critical state of temporal complexity in neural population dynamics, often characterized by a small or near-zero maximum Lyapunov exponent, where input patterns can persist for long durations.
Although these two phenomena are often discussed together and share similar features in information processing, their relationship has not been fully elucidated. Specifically, while some theoretical studies have investigated the conditions for their coexistence, no experimental studies have examined whether they actually coexist. This is because the two phenomena focus on neural activity at different scales. Neuronal avalanches are a phenomenon related to spiking activity—the micro-temporal scale of individual neurons—whereas the edge of chaos is a phenomenon concerning the firing rate of neuronal populations, which is a spatially and temporally macroscopic phenomenon.
In this study, we analyzed publicly available calcium imaging data from the mouse visual cortex to provide the first evidence that neuronal avalanches and the edge of chaos coexist in vivo. By performing a theoretically-grounded coarse-graining, we connected different scales and identified neuronal avalanches and the edge of chaos within the same dataset. Furthermore, we

discovered that efficient power-law encoding, a topic that has been studied in recent years, also occurs simultaneously with these two critical phenomena. Using a mathematical model, we demonstrated that this tripartite coexistence can be realized through biologically plausible synaptic weight distributions. This study integrates two kinds of criticalities and efficient information encoding in the brain and contributes to a comprehensive description of the critical brain hypothesis.

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Title: Decomposing spiking neural networks with Graphical Neural Activity Threads

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Abstract: A satisfactory understanding of information processing in spiking neural networks requires appropriate computational abstractions of neural activity. Traditionally, the neural population state vector has been the most common abstraction applied to spiking neural networks, but this requires artificially partitioning time into bins that are not obviously relevant to the network itself. We introduce a distinct set of techniques for analyzing spiking neural networks that decomposes neural activity into multiple, disjoint, parallel threads of activity. We construct these threads by estimating the degree of causal relatedness between pairs of spikes, then use these estimates to construct a directed acyclic graph that traces how the network activity evolves through individual spikes. We find that this graph of spiking activity naturally decomposes into disjoint connected components that overlap in space and time, which we call Graphical Neural Activity Threads (GNATs). We provide an efficient algorithm for finding analogous threads that reoccur in large spiking datasets, revealing that seemingly distinct spike trains are composed of similar underlying threads of activity, a hallmark of compositionality. The picture of spiking neural networks provided by our GNAT analysis points to new abstractions for spiking neural computation that are naturally adapted to the spatiotemporally distributed dynamics of spiking neural networks.

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Title: Non-topographical contrast enhancement with activation-dependent gain control

Authors: *R. MOYAL, T. A. CLELAND;
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Abstract: Preparing raw olfactory data for subsequent learning and recognition requires extensive preprocessing. For artificial chemosensor arrays and biological olfactory sensory neurons (OSNs) alike, the input consists of a combination of source analytes presented at unpredictable concentrations. To optimally tessellate the input space, receptive fields must adapt their dynamic ranges to observed data distributions. Prior computational work has demonstrated that olfactory bulb circuitry can achieve non-topographical contrast enhancement (NTCE) by combining a half-hat transformation with broad shunting inhibition. In the biological circuit, convergent OSNs excite periglomerular cells (PGs) and external tufted cells (ETs) in parallel, PGs inhibit ETs within columns, and a network of superficial short axon (sSA) cells provides normalizing feedback between columns. We present an improved NTCE algorithm that leverages heterogeneous activation-dependent gain modulation to enrich ET representations, encoding feature ratios independently of the ambient concentration level and making multiscale information available to downstream layers. Specifically, ET cells within each column (and across columns) are subjected to different levels of shunting inhibition as a function of (1) total ensemble activation as mediated by sSA feedback inhibition, (2) neuromodulatory input reflecting a target equilibrium activation sparsity, and (3) the aggregate weight of feedforward inhibition (OSN-to-PG and PG-to-ET synaptic efficacies) affecting each ET cell. The variable sharpening effect of this circuit approximates L^p normalization with different values of p, maximizing the information available to subsequent layers and minimizing feature distortion despite differences in background intensity. We challenge the algorithm using synthetic odor scene data, with feature differences critical for classification spread out across several orders of magnitude. Compared to a homogeneous benchmark, the heterogeneous ET receptive fields better preserve and enhance critical features across scales. Our neuromorphic glomerular circuit, like its biological counterpart, trades off redundancy for expressivity to enable scale-invariant learning in downstream layers. The mechanism is generalizable, and can be deployed as a contrast enhancement solution in any pair of consecutive layers.

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Title: A combination of plasticity rules underlies learning of flexible goal-directed behaviors

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Abstract: Major efforts in neuroscience are dedicated to uncovering synaptic plasticity rules that underlie learning-related changes to neural activity in vivo. Plasticity rules measured in vitro are highly heterogeneous, suggesting that learned behaviors may be supported by specific combinations of such rules. We studied the emergence of preparatory neural activity in the anterior lateral motor (ALM) cortex during a delayed-discrimination task - a paradigmatic example of learned neural dynamics supporting flexible, goal-directed behaviors. We constrained an attractor network model to capture the empirical delay-period dynamics and time-dependent susceptibility to distracting stimuli. Analytical mean-field theory solutions of the network's attractor dynamics revealed that a particular combination of symmetric and temporally asymmetric plasticity rules is necessary to replicate the observed neural dynamics. We extracted the network's effective feedforward connectivity and analyzed its dependence on plasticity rule characteristics. Specifically, we showed that the statistics of chain motifs within this structure are informative of both the plasticity rules in effect and the dynamical features of delay-period neural activity, such as the duration of the transient dynamics. Disrupting these motifs disrupts retrieval dynamics. We extended this analysis to the neural activity cross-covariance and observed similar results. Thus, combined with recent cellular-resolution measurements of functional connectivity in vivo and delay-period activity, our modeling allows us to relate specific changes of network connectivity to the learned behaviors they support. We also performed a stability analysis of the mean-field solutions across different parameter regimes, each of which may represent the network during different stages of 'online' learning implied by distinct curricula. The stability or instability of the learning process may allow us to make testable predictions for the success probability of different learning trajectories. Learned neural activity that emerges based on distinct plasticity mechanisms may facilitate flexible and generalizable assignment of behavioral responses to sensory stimuli. For example, our work may help understand how circuits can, with minimal reorganization, generate the same motor plan in response to different sensory stimuli.

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Poster

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Program #/Poster #: PSTR421.10/XX3

Topic: J.06. Computation, Modeling, and Simulation

Title: Mechanisms governing spatiotemporal activity patterns across neuron populations

Authors: S. SZEIER[1], *H. JORNTELL[2];
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Abstract: At the bottom line, behavior is equivalent to the spatiotemporal pattern of the neuron population activity. Here, we introduce a conceptual framework to describe the network's own governing of its population activity, by using its activity-defining constituents, the weights of the synaptic connections and the current activity of its neurons. We show how a high-dimensional vector field, which describes how the activity distribution across the neuron population is impacted at each instant of time, naturally emerges from these constituents. We show that a mixture of excitatory and inhibitory neurons and a diversity of synaptic weights are critical to obtain a network vector field with a structural richness that allows a constant dynamic evolution in the activity distribution across the neuron population, similar to observations in vivo. The vector field defines an attractor point, towards which the network activity is constantly drawn but can never reach, creating a 'chasing its own tail' scenario. We show that our dynamical system-based framework can capture the context-dependent nature of neural responses and illustrate by example of the corticospinal control of the spinal cord how this results in dynamic, adaptable behavior. This conceptual framework is an alternative to the many current approaches of fitting experimental data observations on neuron population activity to specific models. These approaches often suffer from the inability to generalize to out-of-domain queries. We argue that the vector field approach preserve the structural richness of the data, which in turn is a likely underpinning of the rich behavioral flexibility and adaptability that characterizes biological creatures.

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Title: Hyperflexible and Reconfigurable Neural Circuits

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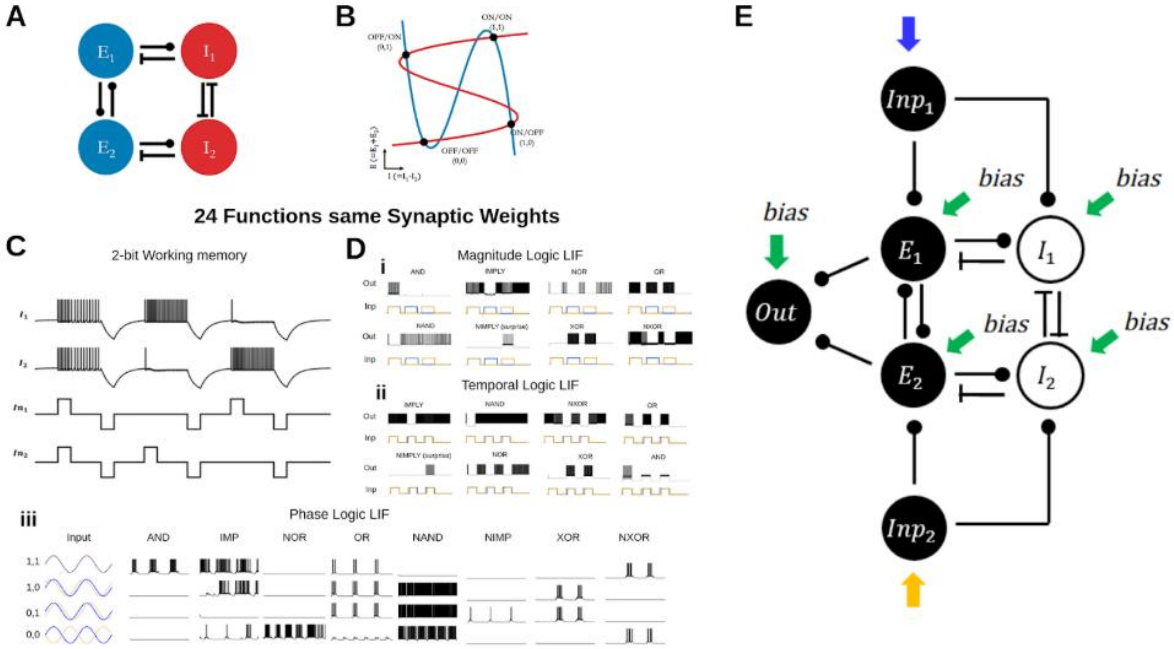
Abstract: Biological neural circuits are remarkably flexible, enabling rapid responses to changing environments. These fast responses occur on timescales too short for synaptic plasticity, suggesting that neural circuits must be inherently reconfigurable. Rather than modifying synapses, circuits dynamically shift into different operational modes, allowing multiple functions to coexist and be selected based on context. This flexibility is believed to arise from the brain's highly recurrent structure, which naturally operates near bifurcation points—where small input changes can cause large, qualitative shifts in behavior.To demonstrate this mechanism, we constructed a 4-neuron recurrent network, CRIREL (Coupled Recurrent Inhibitory and Recurrent Excitatory Loop), capable of compressing 24 distinct functions into a single circuit with fixed synaptic weights. Flexibility in CRIREL emerges from controlling a double-cusp bifurcation, modulated by varying bias currents (constant input currents) and external input, without changing connectivity. These bifurcation-driven transitions produce new stable or unstable states, enabling flexible circuit reconfiguration.We systematically classify the circuit's output across all 8 nontrivial binary logic operations (AND, OR, XOR, NAND, NOR, NXOR, IMP, NIMP), using three input properties relevant to neural coding: amplitude, timing, and phase. Temporal features allow event-driven computations, such as a timing-based XOR where the circuit turns "on" only if spikes arrive ~1 ms apart. All 24 functions coexist in the same network and can be selected purely by adjusting bias currents. We further show that downstream layers using the same architecture can also perform reconfigurable logic through this mechanism.



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Title: Modeling the joint effects of animal movement, adaptation and the environment on hippocampal spatial representations

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Abstract: Place cells in the hippocampus encode the animal's location by increasing their spiking activity within specific spatial regions (place fields). Multiple recent studies characterize place cell activity beyond the simple notion of a firing-rate map of the animal's location within an empty arena. Those include, for example, recording place cells as animals change their movement statistics, characterizing sequences of place cell activity during rest, and recording animals in large, enriched and changing environments. Here our goal is to understand how spatial maps emerge from networks of place cells, by constructing a spatially-coupled spiking network model of neurons in hippocampal area CA3. Mathematical analysis of the population neural activity shows that our model exhibits distinct dynamical regimes, including spatially homogeneous activity, localized activity bumps, and various forms of spontaneously-generated traveling waves. Those regimes can be related to neural activity in vivo, which provides constraints on model parameters. Beyond the transitions between dynamical regimes, we characterize how parameters that quantify running speed, adaptation, neuronal excitability, strength of external inputs and environment size jointly determine place field location and width. We find that when the animal is stationary, place cell activity remains locked to the animal's position. When the animal moves, the model predicts a shift between the encoded and actual locations, which depends on movement speed. The network also exhibits a quiescent state, where a localized subthreshold bump is sustained such that the membrane potential of neurons selective to location close to the actual position is maximal. Together, our model captures key experimental features, including place fields' variability, and their modulation by adaptation, movement and environment size. Owing to the explicit inclusion of an adaptation mechanism which shapes the temporal spiking statistics, our work also provides a framework for analyzing the spatially-modulated inter-spike interval distribution. Those temporal properties, in turn, are crucial for modeling the plasticity of CA3-to-CA1 synapses which supports spatial memory and navigation behaviors.

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Title: Dynamical phases of short-term memory mechanisms in RNNs

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Abstract: Short-term memory, the ability to retain information over seconds, is critical for adaptive behavior, whether tracking a predator's path or recalling a brief transaction. Despite its importance, the neural mechanisms underlying short-term memory remain elusive. Advances in large-scale neural recordings have revealed complex population dynamics, but the rapid, task-dependent reorganization of synaptic connectivity, before and after stimuli or rewards, makes identifying underlying memory mechanisms challenging. Computational neuroscience, by contrast, offers a complementary approach. Controlled simulations enable interpretable models of brain function, and virtual "twins" of neural systems allow researchers to test hypotheses in silico before moving to costly or time-intensive wet-lab experiments. In this work, we perform extensive simulations and identify two distinct attractor mechanisms underlying short-term memory in recurrent neural networks (RNNs): slow-point manifolds and limit cycles. We first characterize these mechanisms using interpretable dynamical systems and low-rank RNNs, then derive theoretical scaling laws predicting which mechanism is learned based on task parameters (e.g., delay duration) and optimization hyperparameters (e.g., learning rate). We validate our framework by training over 80,000 RNNs on delayed-response tasks. Our large-scale experiments reveal that seemingly minor changes in task structure, such as adding a post-response window, can trigger phase transitions between memory mechanisms. Furthermore, the critical learning rate enabling successful memory retention scales as a power law with delay length, with different exponents for each mechanism. Beyond these results, our study has important implications for experimental neuroscience. We show that slow-points and limit cycles can both generate indistinguishable neural sequences during a trial, yet diverge sharply after the task ends. This mechanistic equivalence raises a fundamental question: How can we determine which latent strategy the brain uses if observable activity patterns are the same during behavior? Addressing this challenge may require a shift in experimental design, probing post-trial activity and manipulating task structure to reveal latent dynamical signatures.

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Program #/Poster #: PSTR421.14/Web Only

Topic: J.06. Computation, Modeling, and Simulation

Title: Spontaneous collective oscillation is seen in networks with Hodgkin-Huxley neurons, but not with generalized leaky integrate and fire neurons

Authors: *A. SHEREMET[1], Y. QIN[2], S. ITO[3], J. DAVIS[1];
[2]Civil & Coastal Eng., [1]Univ. of Florida, Gainesville, FL; [3]Allen Inst., Seattle, WA

Abstract: Understanding the relation between the properties of the individual neurons and resulting collective behavior is important for guiding us in selecting the right model. Previously, we have found a largely dismissed type of oscillations, where populations of excitatory Hodgkin-Huxley (HH) neurons exhibit collective oscillations when excited by a Poisson-distributed stationary spike input. Interestingly, this behavior is not universal across various simplified neural models. For example, networks with the Generalized Leaky Integrate and Fire (GLIF) neurons fail to show a similar response. The origin of this discrepancy, and its consequences on the dynamics of large neural populations, are not well understood.
In this study, we investigated the dynamical properties of neural populations modeled with the HH and GLIF point neuron models, with the goal of identifying the dynamical elements responsible for this behavior. We conduct a comparative analysis of the theoretical properties of the neurons, to identify the differences in their major dynamical characteristics. We also establish equivalent domains of their respective parameter spaces, as a basis for comparing their dynamics.
The population response to forcing input is further investigated numerically, using the Brian2 simulators and GLIF models imported from the Modeling ToolKit (BMTK). The neural population is forced by spike trains, either stationary or modulated at a low frequency (theta range), distributed randomly across the population. Our simulations suggest that the HH "susceptibility" parameter "h" plays an important role in creating collective oscillations, potentially pointing toward identifying a broad class of neurons that can exhibit such spontaneous oscillations.
Our work highlights potential mechanisms underlying different origins of the oscillatory activity in neural networks. It also emphasizes the importance of model selection for accurately representing specific types of oscillatory activity. This is particularly important for studies on oscillatory properties of the neural network, especially for realistic representation of interaction of oscillations in multiple frequencies (cross-frequency coupling).

Disclosures: A. Sheremet: None. **Y. Qin:** None. **S. Ito:** None. **J. Davis:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.01/XX7

Topic: J.08. Methods to Modulate Neural Activity

Support: NSERC

Title: Investigating the role of cAMP as a critical regulator of CA1 neuronal population activity in mice during hippocampus-dependent spatial learning & memory

Authors: *J. RAI[1,2], H. LI[2], J. RAI[1], P. ZAWISTOWSKI[2], M. ZHEN[2,1], K.-I. OKAMOTO[2,1]; [1]Univ. of Toronto, Toronto, ON, Canada; [2]Lunenfeld-Tanenbaum Res. Institute, Mount Sinai Hosp., Toronto, ON, Canada

Abstract: The spatiotemporal organization of neuronal population activity is critical for hippocampus-dependent learning & memory. However, the molecular mechanisms underlying the coordination of CA1 neuronal activity and their task-related dynamics remain unclear. Here, we investigate the role of cAMP (adenosine 3′,5′-cyclic monophosphate) signaling to regulate CA1 neuronal activity during hippocampus-dependent spatial learning & memory by cAMP manipulation and fiber-based calcium imaging in freely-behaving mice. cAMP is a ubiquitous secondary messenger that mediates sensory transduction and neuromodulation. We previously reported a rapid cAMP function to enhance the spatial propagation of depolarizing field potentials across murine hippocampal slices, suggesting cAMP may serve to enhance neuronal population activity for memory. To characterize the role of cAMP for hippocampus-dependent spatial learning & memory, we virally expressed the light-sensitive enzyme PAC (Photoactivatable Adenylyl Cyclase) or constitutively active enzyme PDE4cat (Phosphodiesterase 4) in CA1 neurons in freely-behaving mice for cAMP enhancement or suppression, respectively, during the object location test (OLT) and contextual fear conditioning (CFC) test. In the OLT, we observe that cAMP suppression results in a significant memory deficit while cAMP enhancement during training (but not testing) promotes the displaced object preference, indicating a cAMP function critical for encoding of short-term spatial recognition memory. In the CFC test, we utilize a Dox inducible activity-dependent expression system to specifically express PAC or PDE4cat in CA1 neurons activated during CFC. After 24h, we assayed the effect of cAMP manipulation during exposure to the spatial environment (context). We found a significant reduction in freezing behaviour with cAMP suppression compared to control, suggesting that cAMP is critical for context-dependent fear memory retrieval. To directly examine how cAMP may affect CA1 population activity during OLT and CFC test, we recorded the CA1 neuronal response profiles by in vivo calcium imaging in freely-behaving mice. We found similar frequency and duration of neuronal calcium activities between the control and cAMP suppression groups, however, the organization of latent neuronal population states was perturbed by cAMP suppression, suggesting that cAMP may regulate CA1 population activity underlying spatial memory. Further, we will discuss our approach for combined calcium imaging and cAMP optogenetics in the same animal to directly interrogate the effect of cAMP perturbation on CA1 population dynamics during learning & memory.

Disclosures: J. Rai: None. **H. Li:** None. **J. Rai:** None. **P. Zawistowski:** None. **M. Zhen:** None. **K. Okamoto:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.02/XX8

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R01DA029639-12
NIH Grant RF1AG079269

Title: Protein language-model guided opsin engineering yields unorthodox mutations with enhanced photocurrent properties

Authors: *S. EHRLICH[1], A. VANDELOO[2], S. SINHA[3], A. YANG[4], E. S. BOYDEN[4], C. R. FOREST[2];
[1]Mechanical Engin., [2]Georgia Inst. of Technol., Atlanta, GA; [3]McGovern Inst. of Brain Res., [4]MIT, Cambridge, MA

Abstract: Opsins are light-sensitive ion channels used to modulate activity in neurons, with channelrhodopsins such as ChrimsonR enabling precise, wavelength-tuned control allowing for the nondestructive interrogation of neuronal networks. However, engineering opsins with enhanced functional properties remains a major challenge, often requiring labor-intensive screening or prior structural knowledge. Here, we test whether a general protein language model—trained only on naturally occurring protein sequences and without opsin-specific supervision—can effectively guide opsin engineering. Using the Evolutionary Scale Modeling (ESM-1b/1v) framework, we input the wild-type ChrimsonR amino acid sequence and extracted single-residue substitutions with high evolutionary likelihood. 17 mutations were recommended by the models. These mutations were synthesized and expressed in HEK293 cells, followed by whole-cell patch clamp recordings to assess functional impact . Of these, 15 had measurable photocurrent at wavelengths varying from 395 to 635nm. Among these, the model proposed a set of unorthodox mutations at position E300, including E300G and E300P—substitutions not commonly found, or expected, in natural channelrhodopsins. Both mutations showed a marked increase in sustained photocurrent relative to wild-type ChrimsonR (E300G: 305 ± 111pA SEM, E300P: 181 ± 19pA SEM, control: 144 ± 32pA SEM, n=6 for each). Data further suggest that these variants exhibit slower off-kinetics and potentially increased peak currents, though complete kinetic and spectral characterization is ongoing. These gains were achieved without providing the model any structural, photophysical, or functional information. Protein language models open new avenues for the rapid and scalable design of next-generation optogenetic actuators with tailored electrophysiological properties, enabling more precise interrogation of neural circuits across diverse experimental systems.

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Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

Location: SDCC Halls B-H

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Program #/Poster #: PSTR422.03/XX9

Topic: J.08. Methods to Modulate Neural Activity

Support: HHMI Freeman Hrabowski Scholars Program
The Schaefer Research Scholars Program

Title: Shining light on the developing brain: A minimally invasive transcranial optogenetic approach for neural circuit manipulation in pre-weaning mice

Authors: *C. R. MCDERMOTT[1], D. ZELMANOFF[1,2], B. SLOBODA[3], O. YIZHAR[2], B. J. MARLIN[4];
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Abstract: Establishing causal links between neural activity and behavior in pre-weaning mice remains a major challenge in neuroscience, primarily due to limited tools for precise manipulation in the neonatal rodent brain. To address this gap, we developed a minimally invasive transcranial optogenetic approach that permits deep and prolonged photoinhibition of neural activity in untethered, freely behaving pre-weaning mice. This approach leverages a red-shifted, light-sensitive mosquito-derived inhibitory opsin, eOPN3, in combination with a 5 mm glass window implanted over the intact skull. Our ongoing preliminary studies are utilizing this approach to demonstrate its ability to modulate fear learning in pre-weaning mice. Specifically, C57BL/6J wildtype mice receive bilateral hippocampal injections of eOPN3 or GFP (control) on postnatal day 0, followed by intact skull window implantation on postnatal day 12 to allow optical access without craniotomy. After two-days of recovery, mice undergo a three-day fear conditioning paradigm with continuous red LED illumination to activate eOPN3-mediated neuronal inhibition in the hippocampus. We hypothesize that eOPN3-injected mice will exhibit reduced freezing behavior during the retrieval trial 24 hours later compared to GFP-injected controls, thereby demonstrating the utility of transcranial optogenetics for behavioral modulation in pre-weaning mice. Our long-term goal is to leverage this technique to manipulate specific neuronal populations during early-life fear conditioning to identify the circuits that mediate adaptive stress responses in the pre-weaning period.

Disclosures: C.R. McDermott: None. **D. Zelmanoff:** None. **B. Sloboda:** None. **O. Yizhar:** None. **B.J. Marlin:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.04/XX10

Topic: J.08. Methods to Modulate Neural Activity

Support: Gatsby Charitable Foundation 644 (GAT3755)
Wellcome (219627/Z/19/Z)

Title: Low-cost open-source wireless random-access opto-stimulation

Authors: *F. D. YANG, J. LI, J. C. ERLICH;
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Abstract: Optogenetics techniques have been widely adopted in system neuroscience to manipulate neural activity for its precise temporal control, reversibility, and localized effect. Laser-galvo optogenetics systems (blue laser steered by galvanometric scan mirror) have been developed to provide flexible transcranial photostimulation to probe the functional roles of dorsal cortical regions. However, the laser-galvo technique is limited to head-fixed animals, and is expensive and technically challenging to deploy. To provide a more accessible solution we have developed `zapit-LED' and `OptoGrid', two cranial implant systems designed to enable flexible transcranial photostimulation in freely moving (or head-fixed) mice. Zapit-LED features a 64-LED flexible matrix covering the entire dorsal cortex of a mouse, powered and controlled by wireless and battery-free induction technology. Each LED can be individually controlled, and a programmable stimulation sequence using any of the LEDs can be triggered with a latency of 11ms, making the system suitable for neuroscience applications requiring precise temporal control. OptoGrid inherits the 64-LED stimulation capacity but integrates Bluetooth and an easy-to-swap onboard battery to further enable wireless operation in larger arenas and multi-subject experimental setups. We also present behavioral results from freely moving mice that validate the effectiveness and spatiotemporal resolution of our optogenetic stimulation system. As neuroscientists, we designed this system with a strong emphasis on usability and cost-effectiveness to facilitate adoption by the broader research community. Both the hardware and software components of zapit-LED and OptoGrid are open-sourced via our GitHub repository, along with detailed guidance for low-cost fabrication and deployment of the implants.

Disclosures: F.D. Yang: None. **J. Li:** None. **J.C. Erlich:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.05/XX11

Topic: J.08. Methods to Modulate Neural Activity

Support: Funded by NextGenerationEU under the Italian National Recovery and Resilience Plan (PNRR) within the framework of PRIN 2022 PNRR project “ESTROSA: Energy-autonomous System for TReatment of Obstructive Sleep Apnea” (CUP: J53D23014050001).

Title: Design and in-silico validation of an implantable, battery-free, and wirelessly-controlled optoelectronic system for the application in a mouse models of obstructive sleep apnea

Authors: *S. BASTIANINI[1], E. AUGELLO[2], G. BATTISTINI[2], C. BERTEOTTI[1], V. LO MARTIRE[1], D. CORACI[1], M. LOPS[2], E. MIGLIORANZA[1], G. PAOLINI[2], A. SILVANI[1], E. VOLINO[1], A. COSTANZO[2], G. ZOCCOLI[1];

[1]Dept. of Biomed. and Neuromotor Sci., [2]Dept. of Electrical, Electronic and Information Engin. "Guglielmo Marconi", Univ. of Bologna, Bologna, Italy

Abstract: Obstructive Sleep Apnea (OSA) is characterized by upper airways obstruction that may be favoured by tongue muscle hypotonia during sleep. We have previously shown that mice may develop OSA-like events during REM sleep. Optogenetic modulation of tongue muscle motoneurons during sleep may represent a novel, proof-of-principle approach to OSA testable in mice. However, optical fibers physically connected to external light sources may significantly impact on mouse sleep behaviour and are technically challenging inside a whole-body plethysmograph for continuous monitoring of breathing. To solve this issue, we aimed to design a Wireless Power Transfer (WPT) system based on a subdermal device that allows testing optogenetic therapeutic applications on OSA mouse models. This system relies on an inductive coupling operating at the frequency of 13.56 MHz. It features a transmitter (TX) multi-coil array integrated into the semi-cylindrical wall of an experimental chamber (height and radius = 50 mm). The TX array consists of 5 series-connected coils (each consisting of 2 rectangular turns), 4 of which are identical and distributed on the lateral wall, plus a circular coil (35-mm-radius) lies on the chamber floor. The receiver (RX), implantable in the mouse, consists of two identical rectangular coils of 5 turns each, with dimensions of 14 x 15 mm2. The shape, size, and arrangement of the TX and RX coils have been electromagnetically optimized to ensure both an omnidirectional and uniform magnetic field distribution inside the chamber, and the maximization of harvested power regardless of the mouse's movements or orientation, for reliable operation of the selected optogenetic LED (wavelength = 470 nm). Both TX and RX devices are fabricated using planar technology, with the employment of a 35-µm-thick copper metallization deposited on a 25-µm-thick Kapton substrate. The system has been first simulated and optimized using circuit simulation software to meet the power consumption requirement of the selected LED. Then, the fabricated prototype has been tested with the RX device moving in six different positions with a transmitted power of about 740 mW. The worst-case scenario corresponds to the RX device at the edge of the TX system, far from the lateral coils and significantly misaligned with the one on the floor. Here, the measured rectified power available is 14.7 mW, confirming that the system consistently meets the power demands and can maintain uninterrupted LED operation for effective optogenetic neurostimulation, even under the most challenging positioning and orientation conditions of the mouse.

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Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.06/XX12

Topic: J.08. Methods to Modulate Neural Activity

Support: RS-2022-NR070876
RS-2022-NR067861

Title: A Wireless, Microscale Photovoltaic-LED Hybrid Neural Stimulator for Optogenetic Applications

Authors: *Y.-K. SONG[1,2], N. CHOI[1], M. KANG[1,3];
[1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Res. Inst. for Convergence Sci., Seoul, Korea, Republic of; [3]Samsung Electronics Co. Ltd. Suwon, Gyeonggi-do, Korea, Republic of

Abstract: Optogenetics has revolutionized neuroscience by enabling precise, targeted neural modulation through genetically encoded light-sensitive proteins. Despite its potential, the method commonly relies on invasive optical probes, limiting practicality due to tissue damage and complications associated with percutaneous connectors. This study introduces an advanced, wireless, and fully implantable optoelectronic neural stimulator specifically designed for efficient optogenetic applications. The device directly integrates a microscale photovoltaic (PV) cell and a micro-light emitting diode (LED) to circumvent traditional limitations, providing targeted illumination with minimal invasiveness. The stimulator employs a gallium-arsenide (GaAs)-based photovoltaic cell with a tandem structure comprising three serially connected p-i-n junctions optimized for infrared (IR, 850 nm) absorption. This cell efficiently harvests IR light, demonstrating a high power conversion efficiency exceeding 35%. The generated electrical energy powers a blue micro-LED emitting at 450 nm, precisely matching the activation wavelength for Channelrhodopsin-2 (ChR2), a widely used optogenetic actuator. Utilizing standard semiconductor microfabrication techniques, the PV cell and LED are flip-chip bonded, encapsulated in a transparent biocompatible polymer, and fabricated into an ultra-small cube measuring approximately 400 µm per side. Experimental evaluations confirm the stimulator's functional efficacy using ChR2-expressing HEK293 cells and primary cultured neurons from rat hippocampus. The device successfully converts IR input into sufficient blue illumination to trigger ChR2-mediated photocurrents effectively, comparable to conventional optogenetic stimulation setups. Specifically, the experiments demonstrate intensity-dependent neural activation, validating the stimulator's capability for precise neural modulation without reliance on bulky optical fibers or invasive connectors. This innovative microscale stimulator represents a significant advancement in optogenetic technology by enhancing spatial precision and operational efficiency, thus facilitating deeper and more precise brain-region targeting. It holds substantial promise for clinical and research applications aimed at unraveling complex neural networks and developing therapies for neurological disorders. The development of such a minimally invasive, highly efficient optoelectronic neural stimulator significantly extends the potential and practicality of optogenetics for comprehensive neural circuit analysis and therapeutic interventions.

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Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

Location: SDCC Halls B-H

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Program #/Poster #: PSTR422.07/YY1

Topic: J.08. Methods to Modulate Neural Activity

Title: A mri compatible carbon based fiber neural interface for multimodal electrophysiological and neurochemical sensing

Authors: *W. JEON;
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Abstract: The integration of neuroimaging, optogenetics, and electrophysiological techniques remains a key challenge in neuroscience, where high resolution whole brain activity mapping must be reconciled with the need for localized, direct monitoring of neural signals. Functional magnetic resonance imaging (fMRI) offers non invasive, whole brain coverage of hemodynamic responses but lacks the temporal resolution and specificity required for understanding fast neural dynamics or neurochemical events. Conversely, electrophysiological recordings and neurotransmitter sensing with optogenetics provide direct access to neuronal activity and chemical signaling at precise locations but are limited in spatial coverage and often incompatible with MRI environments due to signal artifacts caused by metallic components.To address this gap, we present a multifunctional, fMRI compatible carbon based fiber neural interface from thermal drawing process that bridges these modalities. Utilizing the tissue like magnetic susceptibility of carbon materials, our interface significantly reduces MRI artifacts, enabling artifact free, whole brain imaging during fMRI. Simultaneously, its high electrical conductivity and electrochemical property with optogenetic approach supports real time electrophysiological recordings and neurotransmitter detection, offering localized, high fidelity insights into brain function. The flexible and biocompatible design ensures minimal tissue disruption and long term implantation stability, making it suitable for chronic studies.This platform represents a major step toward fully integrated neurotechnologies, allowing researchers to combine indirect, whole brain imaging with direct, localized sensing of electrical and chemical brain activity. By overcoming key limitations in current multimodal approaches, our device opens new avenues for both basic neuroscience research and translational clinical applications.

Disclosures: W. Jeon: None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.08/YY2

Topic: J.08. Methods to Modulate Neural Activity

Support: EY030441

Title: Selective Optogenetic Control of Frontal Eye Field Inhibitory Circuits in Non-Human Primates Using Red-Light Sensitive ChRmine

Authors: *P. YAZDAN SHAHMORAD[1], L. BUN[2], G. D. HORWITZ[3];
[1]university of washington, seattle, WA; [2]Bioengineering, [3]Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Optogenetic activation of inhibitory neurons provides a powerful means for rapid and reversible cortical inactivation. This technique has be applied to non-human primates but only rarely and then with opsins that are activated by blue light, which is strongly attenuated by brain tissue and dura mater. To establish a more robust and powerful method for reversible cortical inactivation in primates, we used AAV(pHp.eB)-mDLX5/6 viral vectors, which preferentially target inhibitory neurons, to deliver the gene for the red-sensitive channelrhodopsin, ChRmine. We injected this vector into the macaque frontal eye field (FEF), a region controlling eye movements and visual attention. Electrophysiological recordings using single-unit electrodes or Neuropixels probes demonstrated that optical stimulation through the intact dura produced robust excitation of putative inhibitory neurons and suppression of putative excitatory neurons throughout the cortical depth, confirming efficient transduction and light penetration. To validate FEF targeting, muscimol inactivation at the viral injection site produced profound deficits as expected from previous FEF studies. Behavioral testing during visually-guided saccade tasks revealed small but significant effects when optical stimulation was applied during target presentation, including hypometric saccades and increased reaction times for contraversive targets. These effects persisted in memory-guided tasks using the same stimulation timing, suggesting modulation of both target representation and movement execution components. Additionally, some direction-selective neurons exhibited tuning shifts during optical stimulation, demonstrating that local inhibitory circuits shape movement fields. ChRmine functionality remained stable over the recording period (>1 year). Despite robust neurophysiological effects, behavioral effects were much smaller than those observed with muscimol injection. This approach offers a new tool for studying the causal role of specific neural populations in behavior with high spatiotemporal precision, while overcoming key limitations of traditional optogenetic methods by allowing less invasive optical stimulation through the intact dura across multiple experimental sessions

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Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.09/YY3

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH Grant 5R01NS123054-04

Title: A wireless LED driver and recording system for configurable subdural optogenetic arrays in freely-moving nonhuman primates

Authors: *M. SORENSON[1], J. RUDOLPH[1], S. RAUT[2], R. DONG[3], F. SOLZBACHER[4], S. TREMBLAY[5];
[2]Res. & Develop., [1]Blackrock Neurotech, SALT LAKE CITY, UT; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Electrical and Computer Engin., Univ. of Utah, Salt Lake Cty, UT; [5]Univ. Laval / CERVO, Quebec, QC, Canada

Abstract: Optogenetics enables precise, cell-type-specific control of neural circuits but remains underutilized in nonhuman primate models due to challenges in light delivery and chronic implantation. We present a modular, wireless system for subdural optogenetic stimulation and electrophysiologic recording, designed for use in freely-moving macaques. The system supports flexible, chronically implantable 8×8 microLED arrays (64 individually addressable LEDs) with integrated micro-electrocorticography (µECoG) electrodes. These arrays are ultra-thin, biocompatible, and customizable in geometry to conform to cortical surfaces of interest. Local temperature sensors within the array continuously monitor thermal safety during optical stimulation. Control and power are provided by a compact external module (32.5 mm × 32.5 mm × 30 mm) that mounts to a standard Utah Array titanium pedestal. This unit includes a rechargeable battery, USB-C charging, SD card storage, Wi-Fi connectivity, and a multicolor status LED. It receives wireless stimulation commands and streams back recorded neural data, enabling real-time, untethered experimentation. This system directly addresses key barriers to optogenetic research in primates by enabling safe, scalable, wireless neuromodulation and simultaneous recording in a chronically implanted, fully untethered setup. It represents a major step toward translational optogenetic technologies applicable to human therapies for neurological conditions such as focal epilepsy.

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Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.10/YY4

Topic: J.03. Anatomical Methods

Support: DFG grant (ZA990/1-)
ZIAMH002838
ZIAMH002898
ZIAMH002899

Title: Transcranial optogenetic fMRI mapping of the marmoset cerebral cortex

Authors: *D. ZALDIVAR[1], A. R. RIBEIRO GOMES[1], L. IVES[1], N. WRIGHT[4], C. C.-C. YEN[2], L. BAWEKE[1], K. CAMERON[5], M. EPSTEIN-O'ROURKE[6], D. A. LEOPOLD[3]; [1]Section on Cognitive Neurophysiol. and Imaging, [2]Neurophysiol. Imaging Facility, Natl. Inst. of Mental Hlth. Div. of Intramural Res., Bethesda, MD; [3]Section on Cognitive Neurophysiol. and Imaging; Neurophysiol. Imaging Facility, Natl. Inst. of Mental Hlth. Div. of Intramural Res., Bethsda, MD; [4]UCLA David Geffen Sch. of Med., UCLA, Los Angeles, CA; [5]Section of Instrumentation, Natl. Inst. of Mental Hlth., Bethesda, MD; [6]Natl. Inst. of Mental Hlth., Woodinville, WA.

Abstract: Understanding how local circuits within functionally defined cortical areas contribute to large-scale brain networks remains a central challenge in systems neuroscience. Optogenetic fMRI (opto-fMRI) provides a powerful approach to causally probe and map neural circuits, yet its application in non-human primates has been limited. Here, we present a novel approach in the marmoset in which we combine transcranial light stimulation with concurrent fMRI data collection in animals with broad opsin expression across the cerebral cortex. Using a recently described intrauterine viral delivery approach (Ribeiro Gomes et al), we prepared marmosets with one of two excitatory opsins, Channelrhodopsin-2 (ChR2) or ChRmine. Each animal was chronically implanted with a custom chamber giving access to the posterior half of the skull. Optic fibers were positioned over select cortical areas such as MT and DM through a chamber-mounted grid system, enabled focal transcranial optical stimulation during simultaneous whole-brain BOLD fMRI in a horizontal 7T Bruker scanner. The light stimulation paradigm consisted blue (473 nm) or orange (593 nm) wavelengths in a block design (220 pulses per 22s block; 10 ms ON, 90 ms OFF). Preliminary results reveal that targeted transcranial stimulation leads to fMRI activation, both directly underneath the fiber and at remote cortical and noncortical sites receiving anatomical projections from the stimulated region. For example, within area MT, light stimulation of anterior versus posterior subregions led to markedly distinct BOLD activation patterns, with anterior MT stimulation activating frontoparietal regions PE, Opt, MST, and FEF and posterior MT stimulation activating occipitotemporal visual areas V3, TE, V4 and V6. This transcranial optogenetic approach opens new avenues for causal interrogation of mesoscale network dynamics and the functional architecture of the primate brain.

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Poster

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Program #/Poster #: PSTR422.11/YY5

Topic: J.08. Methods to Modulate Neural Activity

Support: DIRP, NIMH, USA, ZIAMH002797

Title: Deviation of Parabolic Avalanches dynamics in Mouse Frontal Cortex during Anhedonia

Authors: *T. SILVA, K. SRINIVASAN, P. KELLS, Y. BIBINEYSHVILI, B. GIFFORD, V. SINFUEGO, C. V. STEWART, T. L. RIBEIRO, D. PLENZ;
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Abstract: Major depressive disorder (MDD) with anhedonia—a reduced ability to experience pleasure—is linked to elevated cortisol and glutamate-related brain damage, particularly affecting the frontal cortex. We investigated how this condition alters brain activity dynamics using a mouse model of depression induced by chronic corticosterone exposure. Adult male mice (N=27) were genetically modified to express the calcium indicator jRGECO1a in the anterior cingulate/medial prefrontal cortex. Using high-speed, high-resolution two-photon imaging, we tracked the activity of ~200 neurons in each mouse for 30 minutes across 50 days. Mice were split into three groups: one received normal drinking water (NO CORT), while the other two received corticosterone in their water (CORT). Anhedonia was assessed using the sucrose preference test and was significantly elevated in the CORT group after three weeks. Following this period, half of the CORT group received a single subanesthetic injection of ketamine (CORT-KET), while the other half received saline (CORT-SAL). Throughout the experiment, the number of active neurons, spike rates, and background noise remained stable across groups. However, pairwise neuronal correlations declined significantly in the CORT group during peak anhedonia, while the NO CORT group showed no such change. Both CORT-KET and CORT-SAL groups exhibited gradual recovery of these correlations over two weeks after corticosterone was stopped. We also observed disruptions in higher-order brain activity patterns known as neuronal avalanches. In the anhedonic state, the power-law scaling relationship between avalanche size and duration was significantly altered but recovered post-treatment. These findings demonstrate that chronic stress-induced anhedonia disrupts both pairwise and network-level brain dynamics in the frontal cortex. Our results suggest that depressive states interfere with the brain's critical state, potentially impairing cognitive and emotional function.

Disclosures: T. silva: None. **K. Srinivasan:** None. **P. Kells:** None. **Y. Bibineyshvili:** None. **B. Gifford:** None. **V. Sinfuego:** None. **C.V. Stewart:** None. **T.L. Ribeiro:** None. **D. Plenz:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.12/YY6

Topic: J.04. Physiological Methods

Support: Fondation pour la Recherche Medicale (SPF202309017717)
Fondation pour l'Audition
Ile-de-France Region (DIM Elicit)

Title: Ultrafast Local Volume of Excitation (ULoVE) with acousto-optic deflectors boosts activation speed and efficiency of single cell resolution optogenetics

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Abstract: Manipulating the activity of neural circuits with single cell resolution is necessary for in vivo dissection of the mechanisms underlying many brain functions. This is currently achieved with parallel illumination techniques, based on digital holography, which spread the excitation light across multiple neurons. However, this approach requires to accumulate large amounts of light energy to reach the action potential threshold. This limits the number of action potentials that can be elicited per unit of time across neurons, setting strong bounds on the size of the neural populations that can be efficiently activated. We reasoned that an ultrafast sequential approach provides higher peak energy levels to each neuron, and thereby would improve activation efficiency due to the quadratic relationship between peak energy and opsin excitation probability with two-photon excitation. To validate this idea, we used ULoVE, an acousto-optic technique previously developed for high-efficiency two-photon voltage indicator imaging (Villette et al. 2019). ULoVE generates local illumination volumes, matching the dimensions of a neuron, and which can be moved from neuron to neuron every 70 µs. When combining this method with the high-conductance opsin ChRmine, bicistronically expressed with GCaMP6m, we could trigger action potentials in a single neuron with a 70 µs illumination duration using as little as 7 µJ of total excitation light in optimized conditions (0.2 µJ laser pulses repeated @ 500kHz). This represents a ~7-fold reduction of the amount of energy per spike compared to the holographic approach with the same opsin (Marshel et al. 2019). Our ULoVE-based approach therefore opens the possibility to stimulate several hundreds to a thousand neurons within tens of milliseconds with no more than 100mW of average laser power, while current techniques can only activate ~100 neurons with similar illumination power over the same time window. We are currently optimizing our new approach by testing further calcium indicator / opsin combinations or expression methods to reduce crosstalk effects observed with the bicistronic ChRmine-GCaMP6m construct.

Disclosures: M. Pisoni: None. **Y. Goulam houssen:** None. **B. Bathellier:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

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Program #/Poster #: PSTR422.13/YY7

Topic: J.08. Methods to Modulate Neural Activity

Support: K. Lisa Yang Brain-Body Center at MIT
Friends of McGovern Fellowship at MIT
McGovern Institute for Brain Research, MIT

Title: Bioelectronic gastrointestinal neurotechnology to investigate the gut-brain axis in feeding behavior

Authors: *R. MONDAL[1], K. PANG[2], P. ANIKEEVA[3], A. SAHASRABUDHE[2], N. BIGLARI[4], E. ALWAY[5], D. V. BOHORQUEZ[6];
[1]Health-Sciences and Technol., [3]Brain and Cognitive Sci., [4]McGovern Inst. for Brain Res., [2]MIT, Cambridge, MA; [5]Neurobio., Duke Univ. Sch. of Med., Durham, NC; [6]Med., Duke Univ., Durham, NC

Abstract: The gut-brain axis is a key regulator of homeostasis and implicated in a wide range of diseases ranging from psychiatric, to metabolic disorders, including diabetes and obesity. Recent studies have highlighted the role of duodenal and gastric vagal sensory neurons that relay stretch information to the brain in reducing food intake upon stimulation. While this finding could offer insights towards a mechanistic understanding of feeding regulation and posits the potential for therapeutic strategies for obesity, investigations are currently limited by a lack of implantable tools to stimulate and record from the rodent gastrointestinal-tract at the end-organ level. Here, we describe an implantable wireless bioelectronic device platform for endorgan interfacing along the gastrointestinal tract. We use this platform to study the role of organ-specific vagal sensory neurons in feeding behaviors. These devices are designed for chronic optogenetic stimulation experiments as well as recordings of GI electromyograms. The proposed cleanroom-free device design and fabrication approach allows for rapid prototyping, is tunable to individual study needs, and puts an emphasis on robustness to enable long-term (~6 week) studies that are often needed to study metabolism and disease etiology. We thoroughly characterize these devices in benchtop set-ups, assess their biocompatibility in vivo, validate the in vivo functionality using acute recordings of vagal and gastrointestinal activity and then utilize them to investigate the differential effects of stimulation of vagal sensory neurons along the GI-tract on feeding behavior.

Disclosures: R. Mondal: None. **K. Pang:** None. **P. Anikeeva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurobionics Inc.. **A. Sahasrabudhe:** None. **N. Biglari:** None. **E. Alway:** None. **D.V. Bohorquez:** None.

Poster

PSTR422: Methods to Modulate Neural Activity: Optogenetic

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR422.14/YY8

Topic: J.08. Methods to Modulate Neural Activity

Title: A SUITE OF AMBIENT-LIGHT-SENSITIVE CHANNELRHODOPSINS

Authors: A. TOSE, A. NAVA, M. J. HODAK, A. R. MARDINLY, *A. NAKA;
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Abstract: Channelrhodopsins are powerful optogenetic tools, but existing options are much less sensitive than endogenous retinal phototransduction. This poses obstacles to therapeutic applications like vision restoration, where there is a pressing need for channelrhodopsins with enhanced sensitivity to low light levels. Historically, advances in improving the sensitivity of excitatory opsins has primarily involved identifying natural variants and refining these via targeted mutagenesis and/or chimerization. While great strides have been made, there have been few large-scale channelrhodopsin engineering campaigns, likely due to throughput limitations imposed by electrophysiological characterization. To address this, we developed an automated patch clamp assay capable of rapidly measuring channelrhodopsin responses during photostimulation across a range of wavelengths and irradiances. We combined this high-throughput platform with machine learning-guided directed evolution to carry out, to our knowledge, the largest channelrhodopsin engineering campaign to date, generating and screening over 1000 unique opsins. From these we have curated a suite of promising new optogenetic tools with altered ion selectivity, accelerated/decelerated kinetics, enhanced photocurrent magnitude, and heightened photosensitivity. Among these are ultrasensitive opsins that exhibit robust photocurrents - capable of driving spiking - when exposed to standard indoor office light. These ambient light sensitive opsins could potentially enable optogenetic gene therapies for vision restoration under everyday lighting conditions, without the need for accompanying medical devices.

Disclosures: A. Tose: A. Employment/Salary (full or part-time): Science Corp. **A. Nava:** A. Employment/Salary (full or part-time): Science Corp. **M.J. Hodak:** A. Employment/Salary (full or part-time): Science Corp. **A.R. Mardinly:** A. Employment/Salary (full or part-time): Science Corp. **A. Naka:** A. Employment/Salary (full or part-time): Science Corp.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.01/YY9

Topic: J.08. Methods to Modulate Neural Activity

Support: Buddhist Tzu Chi Medical Foundation grant number: TCMF-EP 110-02
Buddhist Tzu Chi Medical Foundation grant number: TCMF-MP 113-01-02
Ministry of Science and Technology, Taiwan grant number: 111-2314-B-303-028

Title: Synergistic Effect of Epidural Electrical Stimulation and Exoskeletal Walk Training on Dynamic Balance for Participants with Spinal Cord Injury

Authors: *L.-H. CHEN[1], Y.-C. CHEN[2], S.-T. TSAI[3];
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[3]Neurosurg., Hualien Tzu Chi Hosp., Hualien, Taiwan

Abstract: ***Problem statement:*** Electrical epidural stimulation (EES) or exoskeleton walking training (EWT) is beneficial to individuals with spinal cord injury (SCI). In this study we aimed to evaluate the benefits of combining EES and EWT to manage trunk impairment in individuals with SCI. ***Methods:*** Two participants with cervical SCI (C group) and two with thoracolumbar SCI (TL group) were recruited. Each participant underwent personalized EES and EWT rehabilitation for one month, and the reaching forward task (RFT) was used for pre- and post-intervention assessments. Movement distance and velocity of the center of pressure (COP), and electromyography data (EMG) from the iliopsoas (ILI) and erector spinae (ES) muscles were collected to assess trunk stability and movement function. ***Results:*** Under EES stimulation, EMG analysis revealed increased activation in ILI and ES muscles. Most participants showed new or enhanced activity, although individual responses varied. The moving distance and velocity of the COP increased after one month of EES and EWT rehabilitation, with a significant rise in moving distance. In the subsequent stratification analysis, the moving distance of the C group increased significantly with or without EES ($p = 0.046$). ***Conclusions:*** The synergistic effect of EES and EWT may provide effective rehabilitation for individuals with SCI, particularly those with cervical SCI, helping them regain trunk stability and movement function within one month.

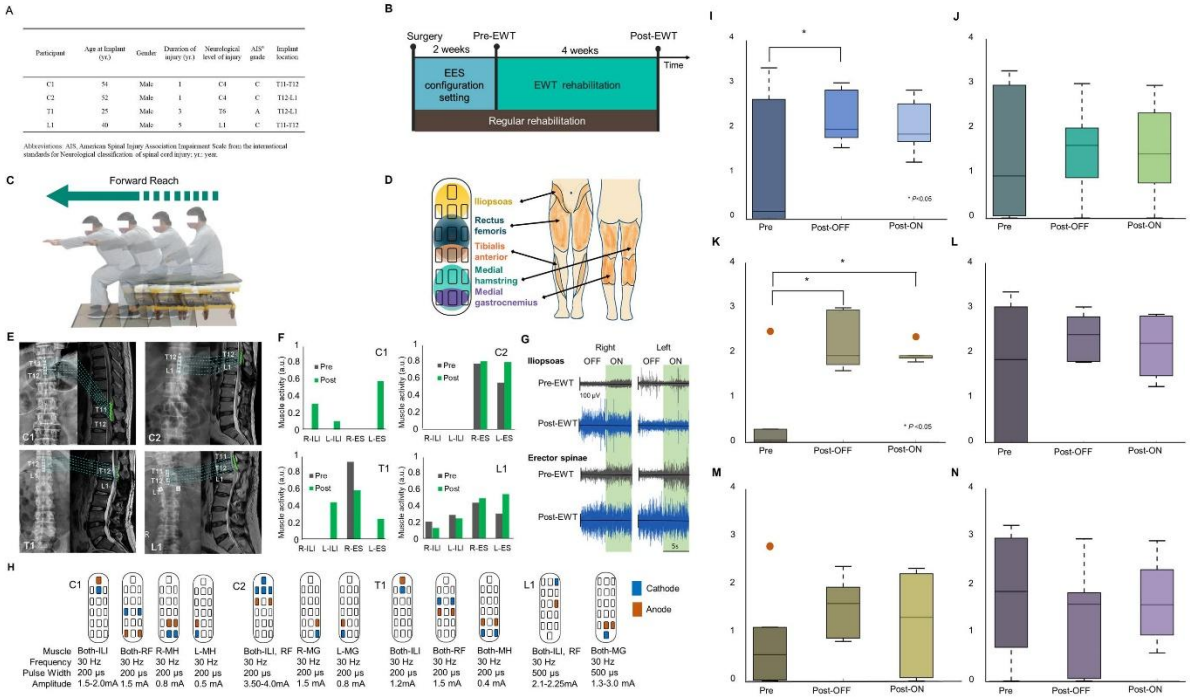

Figure. (A). Characteristics of participants, (B) The experimental design, (C). Reaching forward task, (D) Electrical epidural stimulation, (E) Post-operative images, (F, G) EMG -related metrics and (H) EES setting (I) Moving distance of all participants. (J) Moving velocity of all participants. (K) Moving distance of cervical SCI participants, (L) Moving distance of thoracolumbar SCI participants, (M) Moving velocity of cervical SCI participants , and (N) Moving velocity of thoracolumbar SCI participants

Disclosures: L. Chen: None. **Y. Chen:** None. **S. Tsai:** None.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR423.02/YY10

Topic: J.08. Methods to Modulate Neural Activity

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Title: Real-time bayesian optimization for the fast identification of muscle-selective protocols for epidural electrical stimulation of the spinal cord in human subjects

Authors: *V. FOSSATI[1,2], L. ALBANO[3,2], D. EMEDOLI[4,2], F. AGNESI[1,2], S. IANNACCONE[4,2], P. MORTINI[3,2], S. MICERA[1,5,2], S. ROMENI[2];
[1]Scuola Superiore Sant'Anna, Pisa, Italy; [2]Modular Implantable Neurotechnologies Lab., Milan, Italy; [3]Neurosurg. and Gamma Knife Radiosurgery Unit, [4]Dept. of Rehabil. and Functional Recovery, IRCCS Ospedale San Raffaele, Milan, Italy; [5]Translational Neural Engin. Laboratory, Neuro-X Inst., Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: Epidural electrical stimulation (EES) of the spinal cord has recently emerged as an intervention for restoring movement in patients with spinal cord injury. High inter-subject variability in spinal innervation patterns requires personalized stimulation protocols to effectively restore functional movements. Such optimal protocols are typically identified through manual tuning of stimulation parameters, which is a lengthy and potentially suboptimal process. In this study, we present a Gaussian Process Bayesian Optimization (GPBO) approach to automatically optimize muscle-selective stimulation protocols. We tested the method in real-time in three patients with thoracic motor complete SCI implanted with a 32-contact EES paddle between T11 and L1. The algorithm identified optimal monopolar and bipolar protocols by exploring candidate amplitudes and contact locations. Each candidate stimulation protocol was administered as biphasic cathodic-first pulses at a frequency of 2Hz. Resulting posterior root-muscle reflexes were measured via electromyography (EMG) from eight gait-related muscles on each lower limb. Muscle recruitment was quantified as the peak-to-peak EMG response to a single pulse, normalized to the maximum response from prior calibration. The objective function was a selectivity measure defined as the ratio between the squared recruitment of the target muscle and the sum of all muscle recruitments. This measure, introduced in previous work from our group, maximizes activation of the target muscle, while minimizing activation of non-target ones. The algorithm identified selective monopolar protocols across different target muscles within 25 observations, representing less than 5% of the parameter space. Multiple independent reinitializations yielded consistently robust protocols. Despite sampling only a small subset of the parameter space, the Gaussian Process exploited by the algorithm reliably reconstructed selectivity maps across the full space. Optimization of bipolar stimulation configurations reached satisfactory protocols within 40 iterations, even considering the large increase of the search space introduced by adding the selection of anode electrodes. Optimized bipolar protocols were stable across reinitializations but showed marginal selectivity improvement with respect to monopolar configurations. These results demonstrate the feasibility of using GPBO for fast, real-time inference of optimal EES parameters. Rapid parameter tuning could enable efficient therapy design and fast neuroprosthesis recalibration, thus fostering widespread clinical adoption of neurotechnologies.

Disclosures: V. Fossati: None. **L. Albano:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Università Vita-Salute San Raffaele. **D. Emedoli:** None. **F. Agnesi:** None. **S. Iannaccone:** None. **P. Mortini:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Università Vita-Salute San Raffaele. **S.**

Micera: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EPFL. **S. Romeni:** None.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.03/YY11

Topic: J.08. Methods to Modulate Neural Activity

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ADJOINT2 project N. PR23-PAS-P4 funded by INAIL (Istituto Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro)

Title: Targeted microneurography and intra-neural microstimulation of single-unit tactile fibers in the human median nerve

Authors: *M. STATACHE[1], S. BALLANTI[1], G. D'ALESIO[1], N. FRAGAPANE[1], M. BELLINI[2], S. DALISE[2], C. CHISARI[2], S. TADDEI[3], M. FILOSA[1], C. ODDO[1];
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Abstract: Microneurography (MNG) and intra-neural microstimulation (INMS) are advanced and delicate techniques enabling direct access to peripheral nerve fibers and their functional interrogation. This work presents a method for identifying, recording, and selectively stimulating single-unit tactile mechanoreceptor fibers within the human median nerve with biomimetic patterns. By revealing how tactile information is naturally encoded, these techniques pave the way for restoring a sense of touch in individuals with limb loss. The ultimate goal is to develop neuroprosthetic systems capable of delivering precise, life-like sensory feedback, significantly improving the functionality and embodiment of prosthetic limbs for amputee patients. The protocol involves a setup integrating ADInstruments equipment - PowerLab 8/35, NeuroAmp EX, and Stimulus Isolator - controlled via LabChart software. Under medical supervision, we locate the median nerve at the wrist, using external stimulation with relatively high-current pulses (up to 1 mA, 0.2 ms pulse width). Once the insertion point is identified, a tungsten microelectrode is then inserted into the nerve while a reference is placed subcutaneously. By alternating electrical stimulation with gradually increasing current pulses (starting from 0 mA) and electrode micro-adjustments, we reduce the perception threshold to ~0.02 mA, suggesting proximity to a single afferent fiber or a small cluster of units. Next, MNG is used to refine the

position by monitoring neural responses to natural skin stimulation. Once a single tactile afferent is isolated, we classify it as Slowly Adapting Type I (SAI), Slowly Adapting Type II (SAII), Fast-Adapting Type I (FAI), or Fast-Adapting Type II (FAII) based on adaptation properties, receptive field, and activation threshold. INMS is then employed to deliver neuromorphic spike trains (20-100 Hz variable frequency, 0.2 ms pulses, 0.002-0.01 mA) into the identified fiber. Over 20 participants with no history of neurological or motor impairments (ages 21-40) took part in the study. No gender restrictions were applied. In most cases, we successfully characterized single-unit fibers and mapped their perceptive fields, by observing each single spike evoked when cutaneous mechanical stimulation. In over half the cases, INMS was effectively delivered to single units, eliciting localized, natural sensations of touch or vibration. This method enables direct, selective stimulation of human tactile afferents, supporting the development of bioinspired somatosensory feedback in closed-loop neuroprosthetic systems and providing a powerful tool for studying peripheral neural encoding.

Disclosures: M. Statache: None. **S. Ballanti:** None. **G. D'Alesio:** None. **N. Fragapane:** None. **M. Bellini:** None. **S. Dalise:** None. **C. Chisari:** None. **S. Taddei:** None. **M. Filosa:** None. **C. Oddo:** None.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.04/YY12

Topic: J.08. Methods to Modulate Neural Activity

Support: R21EB032059

Title: Wearable Ultrasound for Real-Time Muscle-Specific Tremor Monitoring in Neurological Disorders

Authors: *N. SHARMA[1], X. XUE[2];
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Abstract: Accurate and continuous monitoring of tremor dynamics is essential for developing personalized, adaptive interventions in neurological disorders such as Parkinson's Disease (PD) and Essential Tremor (ET). Conventional sensing methods, including inertial measurement units (IMUs) and surface electromyography (EMG), lack muscle specificity and suffer from interference due to stimulation artifacts, restricting their use in precise tremor monitoring and intervention strategies. To overcome these limitations, we developed a novel dual-array Wearable Ultrasound Sensing (WUS) system capable of real-time, simultaneous tracking of antagonist muscle dynamics, specifically targeting the flexor carpi radialis (FCR) and extensor carpi radialis (ECR). The developed WUS device comprises two wearable 64-element ultrasound arrays operating at a center frequency of 6 MHz and a -6 dB fractional bandwidth of 50%. In a pilot study involving participants diagnosed with two PD and one ET, standardized tremor-

inducing motor tasks were performed while simultaneously acquiring data from the WUS, commercial B-mode ultrasound, and IMU sensors. Tremor frequencies were identified through spectral analysis of raw ultrasound data converted to M-mode images. Additionally, tissue displacement (TD) was estimated to use a machine learning (ML)-based processing pipeline, significantly reducing computational latency compared to traditional speckle-tracking methods. Statistical equivalence was confirmed through rigorous two one-sided tests (TOST), comparing WUS-derived tremor frequency estimates against established sensing modalities. The results demonstrated that our dual-array WUS accurately detected tremor frequencies, achieving an average error of approximately 10% relative to traditional B-mode ultrasound and IMU methods. The ML-based TD models provided robust predictive accuracy, with correlation coefficients ranging from 0.88 to 0.97, normalized RMSE below 10%, and dramatically reduced training durations. Real-time performance evaluation revealed low frame-level latency (17 ms) and a high processing throughput (>50 Hz), surpassing Nyquist requirements essential for precise tremor tracking. This study demonstrates the viability and effectiveness of wearable ultrasound enhanced by machine learning for precise, continuous tremor monitoring. Our approach offers substantial improvements over existing technologies, laying a foundation for future personalized and closed-loop neuromodulation therapies for individuals affected by tremor disorders.

Disclosures: N. Sharma: None. **X. Xue:** None.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.05/YY13

Topic: J.08. Methods to Modulate Neural Activity

Title: Automated Electrode Identification for Spinal Cord Stimulation Using nnU-Net Segmentation

Authors: *C. V. IHEDIWA[1], S. JONES[2], J. I. HERNANDEZ BELLO[3], A. DALRYMPLE[1]; [1]Biomed. Engin., [2]Univ. of Utah, Salt Lake City, UT; [3]Biomed. Engin., Univ. of Utah, salt lake city, UT

Abstract: Neural injuries such as spinal cord injury and stroke lead to significant motor impairments. Motor neuro-prostheses can improve rehabilitation outcomes and increase independence and mobility. Spinal cord stimulation (SCS) is used for chronic pain management[1] and is under investigation for restoring upper & lower-limb function post-injury for improving gait recovery[2].
Electrode location is determined intraoperatively using fluoroscopy, with confirmation of electrode location relative to the vertebral segments in X-ray images. We aim to correlate anatomical electrode placement with recorded muscle activity. However, current methods of manually identifying lead placement are time-consuming and prone to inaccuracies. This limits the ability to accurately correlate muscle activity with the corresponding site of SSC electrode.

To address this, we propose using image analysis and machine learning methods to automatically identify electrode position relative to vertebral levels.
We reviewed records from 73 patients with SCS implants and generated a dataset of 208 de-identified X-ray images.The leads were placed between T7 and S2. Images were resized to 512×512 pixels, grayscale normalized, and centercropped. Background, vertebrae, and electrodes were manually annotated in each image. The dataset was divided into training (70%), validation (15%), and testing (15%). A modified nnU-Net[3] was trained on the training set,optimized with the validation set, and evaluated on the test set. Data augmentation (30-50% of the test set) included±30° rotation, 0.7-1.4× scaling, gamma shift (0.7-1.5), noise injection, and mirroring. Training occurred over 500 epochs with batch size of 8, cross-entropy loss, and stochastic gradient descent with Nesterov momentum.
The model achieved a Dice score of 87.2% and an overall pixel accuracy of 96.9%. Background and vertebrae are classified with high accuracy (98.3% and 94.7%, respectively), with electrode classification accuracy of 78.6%,possibly due to class imbalance and the small size of the electrodes. The trained nnU-Net will be critical for intraoperative studies recording muscle activity from lower limb muscles during SCS, enabling the correlation of muscle activity to electrode position relative to the anatomy. As SCS expands as a rehabilitation intervention, there is a critical need to link electrode locations to muscle . This work represents a valuable tool for researchers and clinicians aiming to automate the correlation of muscle activity with electrode location.
References: [1]Caylor et al., 2019; [2]Iversen et al., 2024; [3]Isensee et al., 2021.

Disclosures: C.V. Ihediwa: None. **S. Jones:** None. **J.I. Hernandez Bello:** None. **A. Dalrymple:** None.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR423.06/YY14

Topic: J.08. Methods to Modulate Neural Activity

Support: University of Utah Biomedical Engineering Department
SPUR Program at University of Utah

Title: Using the Posterior Root Muscle Reflex to Infer Spinal Cord Excitability in Individuals With Lower Limb Amputation

Authors: *A. HARRISON[1], H. HABIB[3], K. VALESTRINO[2], G. SIMPSON[2], A. DALRYMPLE[2];
[1]Univ. of Utah, salt lake city, UT; [2]Biomed. Engin., Univ. of Utah, Salt Lake City, UT; [3]Biomed. Engin., Wright State Univ., Dayton, OH

Abstract: The Posterior Root-Muscle (PRM) reflex is elicited by electrically stimulating the dorsal roots of the spinal cord using transcutaneous spinal cord stimulation (tSCS). The PRM reflex can be used to infer spinal cord excitability by examining characteristics of the reflex response evoked by tSCS. This study aims to characterize the PRM reflex in healthy, neurologically intact individuals, as well as individuals with a lower-limb amputation to quantify changes in spinal cord excitability that occur after limb amputation. We will be using age-approximated comparisons for these two groups, as previous work has shown that spinal cord excitability decreases naturally with age[1].
PRM reflexes were evoked using a 1 ms, monophasic, cathodic square pulse delivered through an adhesive electrode placed lateral to the T12-L1 vertebrae. A return electrode was placed on the ipsilateral anterior superior iliac spine. PRM reflexes were collected in 26 neurologically-intact participants (13 F, 23-49 yo), as well as three individuals with lower-limb amputation in a previous study (1 F, 36-60 yo)[2]. Electromyography (EMG) sensors were placed over the soleus muscle of the ipsilateral leg, relative to the stimulating electrode, to record the PRM reflex. Stimulation was first delivered at a sub-threshold level, then increased in 5 mA increments until PRM reflex responses first appeared in the EMG recording (threshold), after which the threshold was identified with 1 mA precision. Stimulation was then increased in 5 mA increments until the responses no longer increased in amplitude (maximum).
PRM reflex thresholds in the neurologically-intact group were 35±9.9 mA for ages 20-29 (n=14), 40±19 mA for ages 30-39 (n=5), and 42±7.8 mA for ages 40-49 (n=2). In the individuals with lower-limb amputation, thresholds were 63.4±1.7 for ages 30-39 (n=1), 59.3±3.7 for age 40-49 (n=1), and 55.8±8 for age 50-59. Additional reflex thresholds per decade in the neurologically-intact control group are expected to provide more information about how the PRM reflex changes naturally with age and provide the necessary information to compare with individuals with amputation or other neurological injuries. With additional participants with a lower-limb amputation, we will infer how neural injury alters spinal cord excitability independent of the changes that happen naturally with age.
References: [1] Kido, A., et. al., *Can. J. Physiol. Pharmacol.,* 2004, [2] Dalrymple, A. N., et. al., *J. Neural Eng.*, 2024.

Disclosures: A. Harrison: None. **H. Habib:** None. **K. Valestrino:** None. **G. Simpson:** None. **A. Dalrymple:** None.

Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.07/YY15

Topic: J.08. Methods to Modulate Neural Activity

Title: Modeling of wireless resonant inductive stimulation of peripheral nerves via miniaturized bioelectronic rings

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Abstract: Peripheral nerve stimulation offers powerful therapeutic potential for modulating autonomic and somatic function, yet existing implantable devices rely on batteries, wired leads, or complex surgical procedures. We present a wireless neural interface based on resonant inductive coupling through miniaturized bioelectronic rings designed to encircle peripheral nerves and deliver targeted stimulation without direct electrical connections.
Each ring comprises a conductive loop with a micron-scale circumferential gap, forming an LC resonator. When exposed to an externally applied oscillating magnetic field tuned to its resonant frequency, the structure generates a highly localized electric field at the gap. Finite element simulations (COMSOL) confirm that the resonant configuration amplifies the induced field strength and confines it spatially to the nerve-facing region of the ring, enabling precise axonal depolarization with minimal off-target effects.
This approach eliminates the need for implanted power sources or tethered wires, enabling minimally invasive deployment and long-term use. The system architecture supports scalability for multi-site stimulation and integration with wireless control schemes. Current work focuses on optimizing resonator geometry, maximizing field efficiency, and preparing for in vivo validation. This technology offers a path toward next-generation peripheral neuromodulation interfaces for therapeutic applications in pain, inflammation, and motor control.

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Poster

PSTR423: Advancements in Spinal Cord and Peripheral Nerve Stimulation

Location: SDCC Halls B-H

Time: Wednesday, November 19, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR423.08/YY16

Topic: J.08. Methods to Modulate Neural Activity

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Title: Collective Neural Dynamics of the Spinal Cord Medeled Through a Manifold Based Recurrent Framework

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Abstract: Autonomic functions such as blood pressure regulation arise not from uniform neural responses, but from complex, time-evolving dynamics within distributed circuits. The intermediolateral nucleus (IML) of the spinal cord is a central integrator of sympathetic outflow, yet how its recurrent circuitry encodes physiological state transitions remains poorly understood. Here, we investigated how population dynamics within the IML contribute to reflexive cardiovascular regulation. In vivo electrical stimulation of the IML reliably evoked blood pressure reduction, during which multi-unit recordings captured heterogeneous neuronal responses. Some neurons increased their firing, others decreased, and many remained unchanged. Despite this diversity, dimensionality reduction of the population activity revealed a consistent low-dimensional circular manifold that captured both the temporal evolution of neural trajectories and ongoing blood pressure fluctuations. This latent structure suggested a population-level representation of autonomic transitions not observable from single-unit activity alone. To test whether this manifold emerges from intrinsic circuit computation, we developed a biologically constrained recurrent neural network (RNN)—the IML Circuit Model (ICM)—which embeds the empirical manifold as a dynamic reservoir. By integrating external stimulation with internal latent states over time, the ICM predicted both blood pressure and neural dynamics with over 80% accuracy. Unlike feedforward models, the RNN-based ICM preserved temporal structure through recursive memory, reproducing heterogeneous yet coordinated population responses. Perturbation analysis further demonstrated that the circular manifold actively sustains reflex dynamics, confirming its computational role beyond simple dimensionality reduction. Together, these findings reveal that the IML functions as a recurrent computational unit that encodes and maintains physiological transitions via low-dimensional latent dynamics. The ICM serves as a digital twin of spinal autonomic regulation, offering mechanistic insight into reflexive control and establishing a foundation for precision neuromodulation strategies targeting interpretable neural states.

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Poster

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Title: Design, fabrication and preliminary in vivo investigation of intradural spinal electrode arrays

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Abstract: Spinal cord injuries (SCIs) represent a life-altering event in the lives of affected subjects, resulting in acute medical interventions, lifelong limitations and long-term rehabilitation. Consequently, there is a strong interest in finding therapeutic strategies aimed at mitigating acute complications as well as enabling long-term neuroregeneration and sensorimotor recovery. Spinal Electrode Arrays (SEAs) were designed and developed in a modular manner for the first time using laser structuring technologies. Preliminary studies in Aachen Minipigs were performed to evaluate the in vivo material-tissue interface performance after implantation around the spinal cord. SEAs were designed and fabricated utilizing a silicone-based microfabrication process, with a platinum foil (thickness t_{Pt} = 25 µm) between two layers of medical grade silicone (t_{PDMS} = 250 µm) and structured by laser ablation. The implants are designed as a T-shape with the upper parts acting as wings with a total length of 25 mm, that can be wrapped around the spinal cord. These wings contain 12 electrodes with round openings (diameter d_E = 0.8 mm) and a pitch of 2 mm in between electrodes. With the base of the 'T' acting as contact pads, helically wound cables with 12 strands of MP35N wires and a diameter of 12 µm each were first soldered, then welded onto pads (openings sizes 0.8 x 0.3 mm^2), cleaned and casted with silicone. These implants were inserted in Aachen Minipigs (7-month old) intradurally. Multiple implants without cables served as control samples. Tests with electrochemical impedance spectroscopy (freq. = 1 Hz - 100kHz) proved successful connection of most of the welding joints. Two months after implantation, the spinal cords were extracted from the pigs and analyzed. SEAs with connected cables exhibited strong compression at the contact pads, harming the neighboring tissue. Compression seems more severe after intradural placement, compared to epidural implantation. Control implants without cables yielded good optical results with only minor compressions. SEAs in the used design showed too strong compression on the spinal cord at the contact pads and displayed partially high impedances, which can be traced back to the laser welded connection between SEA and cable. Change in implant design with contact pads more distant to the electrode array site on the spinal cord will be implemented. Further change in the welding technique from laser to resistance welding showed lower impedances and will be considered for future implants.

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Poster

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Topic: J.08. Methods to Modulate Neural Activity

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Title: Meta-analysis of spinal cord stimulation parameters for motor function in Parkinson's disease

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Abstract: Spinal cord stimulation (SCS) has been tested as a treatment for the motor symptoms of Parkison's disease (PD). Available evidence stems mainly from observational non-controlled studies, conducted in heterogeneous conditions, with inconsistent programming of stimulation parameters. To identify stimulation parameters that caused the best therapeutic effect, we conducted a meta-analysis of the studies reporting the motor improvements by SCS performed in Parkinson's patients. The search was performed in the PubMed database with the studies available until April 2025 using "spinal cord stimulation" and "parkinson's" as search criteria. Articles were selected according to the following criteria: original studies with the inclusion of patients with PD who had undergone spinal cord epidural stimulation, with a detailed description of the stimulation parameters, and the use of the UPDRS motor scale. Eighteen studies were selected, encompassing 77 patients. The percentage improvement in UPDRS motor scale was correlated to parameters differing between studies, including spinal level, pulse frequency, pulse width, years since PD diagnosis, and tonic versus burst stimulation paradigm. No significant correlations were observed between any of these parameters and motor improvement across studies, indicating that the parameters typically programmed in tonic and burst stimulation may not be critical for the therapeutic effect of SCS in PD. Future research should thus consider alternative SCS stimulation paradigms, beyond tonic and burst, for alleviating motor symptoms of PD. Such paradigms might include a personalized approach based on electrophysiological biomarkers and/or movement sensors, that takes into account the patient's activity levels and physiological conditions, and should become the future standard for SCS and other neuromodulation treatments for motor disorders.

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Poster

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Title: Effects of redundancy in closed-loop ICMS feedback for neuroprostheses

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Abstract: Intracortical microstimulation (ICMS) has been shown to be an effective method for providing closed-loop sensory feedback in multiple animal models. Previous research has investigated encoding of visual, auditory, and tactile signals, however there is little prior work encoding a proprioceptive signal using ICMS. To identify the most effective methods for encoding proprioception, we have developed a target location task in freely moving mice using ICMS feedback that mimics proprioceptive encoding in primary somatosensory cortex by encoding the direction and distance of the target location. This research has shown that a completely novel signal can be learned to a high degree of utility very quickly, however there remain open questions such as how much redundancy the ICMS signal should carry and if it should sparsely encode relevant stimuli across few electrodes, or broadly encode stimuli across many electrodes. To investigate this question, 2 cohorts of mice were trained initially with either a sparse or broad encoding algorithm before being switched to the other. We found that mice could rapidly switch between broad and sparse encoding algorithms requiring 20-30 trials to acclimate to the new algorithm. We also found that mice performed significantly better with a broad encoding algorithm than sparse when the ICMS signal was presented in conjunction with a static visual target. Interestingly, there were no significant differences in performance between the two encoding algorithms when mice were only provided ICMS feedback to find the target location. These results indicate that under multimodal sensory conditions, a broader encoding algorithm is more useful, however, sparse encoding may be slightly more effective in unimodal conditions. These results seem to match our general understanding of how natural sensation is encoded in the brain under unimodal and multimodal conditions and indicate that future encoding schemes should account for other available sensory stimuli.

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Poster

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Program #/Poster #: PSTR423.12/YY20

Topic: J.03. Anatomical Methods

Title: Methodological considerations for inducing thoracic 8-9 spinal cord injury in rats

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Abstract: Spinal cord injury (SCI) leads to the loss of essential functions such as motor control, sensation, and autonomic regulation. Traumatic SCI is the most common form, and rodent

models, particularly rats, are widely used to study its mechanisms and treatments. Depending on the research goals, different spinal levels may be targeted. An injury at the thoracic 8/9 (T8/T9) level induces paraplegia, hindlimb sensory loss, and dysfunctions in bladder and bowel control. However, achieving a precise T8/T9 lesion requires accurate anatomical identification, which is often insufficiently described in the literature. This work outlines a detailed methodology for inducing T8/T9 SCI in rats. Because spinal cord segments are not aligned with their corresponding vertebrae, the seventh thoracic vertebra (T7) must be removed to access the T8/T9 spinal cord. T7 can be located between the 5th and 6th ribs; however, identifying vertebrae based on rib count is challenging. A more reliable landmark is the T11 spinous process, which is palpably prominent. A skin incision is made from T11 to T5, followed by retraction of skin and muscle. A triangular tendon spanning T9-T11 serves as an additional anatomical reference. After careful dissection and retraction of the muscles from T6 to T8. T7 is exposed and can be removed using a drill or rongeurs until the spinal cord and its roots are visible. The pia mater should remain intact unless experimental design dictates otherwise. Once the spinal cord is sufficiently exposed, a lesion can be performed using transection, compression, or contusion, depending on the study's objectives.

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