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Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: P20GM113131-7006
R15MH126317
R15MH125305
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K.J. by an undergraduate research award at UNH

Title: Reelin Controls the Directional Orientation, Intracellular Positioning and Morphology of Primary Cilia in Pyramidal Neurons of the Cerebral Cortex

Authors: *S. AKTER[1], S. MIRHOSSEINI[2], M. HATTORI[5], M. LYON[3], L. QIU[1], X. CHEN[4]; [1]Molecular, Cell. and Biomed. Sci., Univ. of New Hampshire, Durham, NH; [2]Molecular, Cell. and Biomed. Sci., Univ. of New Hampshire, Dover, NH; [3]Mathematics and Statistics, [4]Dept. of Molecular, Cell. and Biomed. Sci., Univ. of New Hampshire, Durham, NH; [5]Dept. of Biomed. Science, Grad. Sch. of Pharmaceut. Sciences, Nagoya City Univ., Nagoya City Univ., Nagoya, Japan

Abstract: We have previously reported that the primary cilia of pyramidal neurons in inside-out laminated regions orient predominantly toward the pial surface, reflective of a reverse movement for neuronal positioning. However, the mechanisms underlying the cilia directionality and reverse movement are unknown. Here we show that the primary cilia of pyramidal neurons in inside-out laminated regions of wild type (WT) mice are generally positioned at the base of the apical dendrites and aligned on the nuclear side opposite to the axon initial segment. However, this phenomenon is not observed in interneurons, astrocytes, atypical pyramidal neurons in the deep neocortex, and excitatory neurons in the amygdala and other non-laminated brain regions, where their cilia are irregularly positioned within the soma around the nucleus, and cilia lack directional orientation. In Reelin deficient mice (reeler), primary cilia of principal neurons in the piriform cortex and the outer neocortical layers do not exhibit directional orientation. Consistently, their cilia/centrioles are not consistently located at the base of the apical dendrites. The cilia position and orientation in late-born neocortical and CA1 neurons are partially affected by Reelin deficiency. Moreover, loss of Reelin increases cilia length in principal neurons throughout the cerebral cortex, but not in interneurons, astrocytes, or excitatory neurons in non-laminated brain regions. Taken together, by influencing radial migration and afterwards reverse

movement, Reelin controls the directional orientation, intracellular positioning, and morphology of primary cilia in pyramidal neurons in the cerebral cortex.

Disclosures: S. Akter: None. **S. Mirhosseini:** None. **M. Hattori:** None. **M. Lyon:** None. **L. Qiu:** None. **X. Chen:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: RS-2023-00265581
RS-2023-00260454
RS-2024-00353657
24-BR-03-01

Title: Ligand-independent EPHA2 signaling preserves neocortical progenitor identity by sustaining mitochondrial Complex I

Authors: *T. DIEM NGHI, S. SHIN, W. LEE, S. PARK;
Dept. of Life Sci., Pohang Univ. of Sci. and Technol. (POSTECH), Pohang, Korea, Republic of

Abstract: Embryonic neural stem and progenitor cells (NSPCs) occupy a specialized niche alongside the lateral ventricles, where receptors on their apical surface mediate extrinsic cues essential for preserving progenitor identity. However, the intracellular mechanisms, particularly those targeting metabolism, that translate these signals into NSPC fate decisions remain poorly elucidated. We identify a previously unrecognized role for ligand-independent EPHA2 receptor signaling, selectively enriched within NSPCs, in maintaining progenitor self-renewal by preventing premature cell cycle exit. Mechanistically, this signaling triggers protein phosphatase 2A-mediated dephosphorylation of the mitochondrial Complex I assembly factor ECSIT at Thr179, facilitating its mitochondrial localization. This regulatory event sustains Complex I function and supports NSPC metabolic fitness and proliferative capacity. Notably, maternal NAD^+ supplementation rescues progenitor depletion and mitigates aberrant neurogenesis caused by EPHA2 pathway disruption. These findings delineate an EPHA2-ECSIT-mitochondria axis that bridges membrane receptor activation with intracellular metabolism, addressing a fundamental gap in our understanding of progenitor maintenance.

Disclosures: T. Diem Nghi: None. **S. Shin:** None. **W. Lee:** None. **S. Park:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI 24K01923

Title: In vitro supplementation of docosahexaenoic acid provides neuroprotective evidence against oxidative stress in canine glial- and neuron-like cells

Authors: C. MARASIGAN[1,2], Y. OTANI[3], T. ICHIKAWA[4], S. MAEDA[1], Y. MOMOI[1], *T. YONEZAWA[5,1];
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Abstract: The brain is susceptible to oxidative stress due to its high oxygen consumption and lipid-rich composition. In dogs, the persistence of reactive oxygen species contributes to the development of neurodegenerative diseases like epilepsy. Docosahexaenoic acid (DHA) is an essential component of neuronal membranes that functions for neuromodulation affecting signal transduction pathways; however, its production is limited. While neurological investigations often involve murine models, *in vitro* systems for the canine brain are still lacking. Here, we investigated the role of DHA in attenuating oxidative injury using the canine glial-like (J3T1) and neuron-like (J3T2) cells. When both cell lines are exposed to hydrogen peroxide to induce oxidative condition following a 24-hour DHA pre-treatment, we find that DHA retains higher cell viability compared to the vehicle control group. Consequently, we observe that the production of the oxidative stress marker 8-OHdG is reduced in cells treated with DHA. In addition, we quantified the relative gene expressed for heme oxygenase 1 (*HO1*) and peroxiredoxin 1 (*PRDX1*) in both cell lines and found that DHA downregulated their expression, indicating a positive anti-oxidative response. This result also coincides with the comparative expression of *Bax* and *Bcl2* in DHA-treated cells, signifying its potential in preventing cell death. DHA further demonstrated its protective effects by reducing the number of TUNEL-positive cells and by preventing further damage to the actin filaments of J3T1 and J3T2 cells, confirming its protective effects against oxidative injury. Our data suggests that DHA effectively attenuates oxidative damage through its antiapoptotic and antioxidant properties. This neuroprotective evidence provides insights into the actions of DHA in protecting glial- and neuron-like cells undergoing oxidative stress, a key factor in the progression of neurodegeneration.

Disclosures: C. Marasigan: None. **Y. Otani:** None. **T. Ichikawa:** None. **S. Maeda:** None. **Y. Momoi:** None. **T. Yonezawa:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

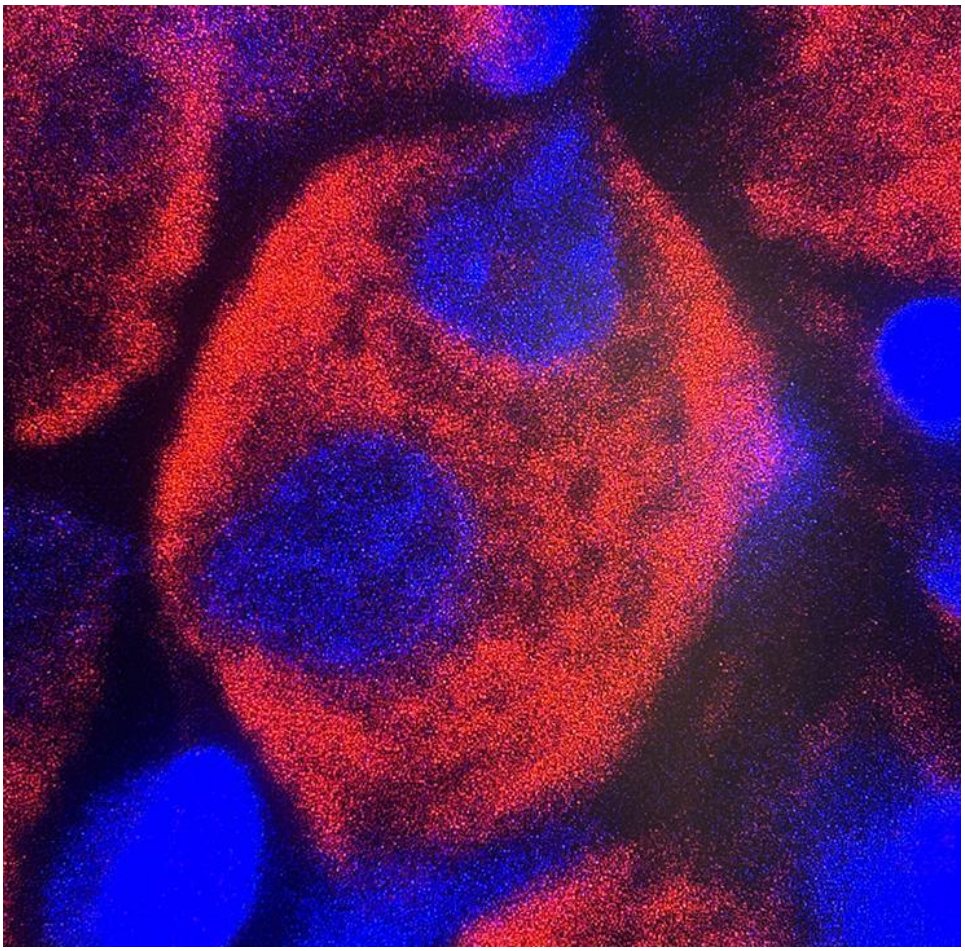
Program #/Poster #: PSTR053.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Title: Citron kinase plays a role in cytokinesis of neural crest derived cells

Authors: J. POPP[1], A. PELLICANO[2], V. GRUBISIC[2], *R. L. RAMOS[3];
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Abstract: Citron kinase (CK) plays an important role in cytokinesis. Not surprisingly, deficits in CK function cause microcephaly in rats, mice, and humans. A characteristic feature of the microcephalic phenotype associated with CK dysfunction includes binucleate and multinucleate cells throughout the central nervous system. Thus, neural tube derived progenitors require CK for normal cytokinesis. In contrast, the role of CK in cytokinesis of neural crest derived cells is less understood. In the present report, we use the CK mutant rat model called "Flathead" to examine several neural crest cell derived structures for the presence of binucleate cells. Our sample includes the trigeminal ganglia, dorsal root ganglia, adrenal medulla, and enteric nervous system. Using several histological approaches and confocal microscopy, we provide evidence that CK function is required for cytokinesis of several neural crest derived cell types. For example, in the attached image we show a binucleate dorsal root ganglion cell labeled with propidium iodide and DAPI to reveal cytoplasmic and nuclear compartments, respectively. Our results are important toward understanding the role of CK function during nervous system and other organ system development.



Disclosures: J. Popp: None. **A. Pellicano:** None. **V. Grubisic:** None. **R.L. Ramos:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant U01DA054170
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NIH Grant R01NS124855

Title: Regulation of neurogenesis and neuronal migration by Rrm2 and Timp3 following seizures

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Abstract: Temporal lobe epilepsy is associated with aberrant neurogenesis and ectopic migration of adult-born granule cells (abGCs), yet the molecular mechanisms driving these changes remain poorly defined. Using a pilocarpine-induced mouse model of temporal lobe epilepsy and chemogenetic silencing of abGCs via Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), we previously demonstrated that abGC inhibition reduces both ectopic migration and seizure susceptibility. To identify underlying molecular regulators, we performed RNA sequencing of FACS-isolated abGCs and identified *Rrm2* and *Timp3* as top candidate genes modulated by seizure activity and neuronal silencing. To assess their functional roles, we pharmacologically inhibited *Rrm2* with Triapine (3-AP) and *Timp3* with the LXR agonist T0901317 (09). Inhibition of *Rrm2* significantly reduced seizure frequency, decreased hilar abGC migration, and altered neuronal differentiation. While T09 treatment did not significantly alter *Timp3* transcript levels, it increased the number of DCX+ neurons in the hilus, suggesting an effect on neuronal migration independent of robust knockdown. These findings highlight distinct roles of *Rrm2* and *Timp3* in hippocampal plasticity following seizures. Together, these data identify new molecular targets for modulating aberrant neurogenesis in epilepsy.

Disclosures: A.O. Adeyeye: None. **P. Varma:** None. **N. Enerlan:** None. **M. Coppin:** None. **J. Hsieh:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: Grants-in-Aid for Scientific Research JP18K06512
Grants-in-Aid for Scientific Research JP21K06405
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Multidisciplinary Frontier Brain and Neuroscience Discoveries JP24wm0625215
The Japan Agency for Medical Research and Development JP24gm1310003
The Japan Agency for Medical Research and Development JP24jm0210097

Title: Radial Glial Cell Cycle Dynamics Shape Subtype-Specific Microcolumns in Neocortical Layer 5

Authors: *Y. NARIYA[1], H. MARUOKA[2], S. OKABE[3];
[1]Kameda Med. Ctr., Kamogawa Chiba, Japan; [2]Next Generation Med. Develop. Res. Core Inst. for Frontier Sci. Initiative, Kanazawa Univ., Ishikawa, Japan; [3]Dept. of Cell. Neurobiology, Grad. Sch. of Med., Univ. of Tokyo, Tokyo, Japan

Abstract: The neocortex integrates information through local microcircuits consisting of small ensembles of neurons organized within limited tangential domains. While these structures are critical for cortical computation, the developmental mechanisms underlying their emergence are not yet fully understood. Our previous studies demonstrated that layer 5 of the adult mouse neocortex contains subtype-specific neuronal clusters, or microcolumns. These microcolumns are regularly spaced in a hexagonal lattice and function as modular circuit units across multiple cortical areas. We further discovered that microcolumn formation is intrinsically regulated by the temporal dynamics of the cell cycle in radial glial cells (RGCs), the primary neural progenitors during cortical development. However, these findings pose a critical question regarding the mechanistic contribution of specific progenitor cell dynamics to microcolumnar organization. In order to address this question, we developed a computational model that simulates microcolumn formation under a range of developmental conditions. We chose seven key parameters based on their roles in cortical development: (1) cell cycle phase offset between neighboring RGCs, (2) phase variability among RGCs of the same subtype, (3) the generation probability of intermediate progenitor cells, (4) the frequency of tangential neuronal migration, (5) the phase specificity of migration events, (6) the initiation timing of axonal anchoring relative to neuron birth, and (7) the total duration of the RGC cell cycle. Our systematic parameter manipulation revealed three critical findings. Firstly, anti-phase cell cycling between adjacent RGCs was imperative for establishing spatial periodicity. Secondly, high tangential migration frequency enabled broader neuronal dispersion, while moderate phase specificity helped preserve columnar boundaries. Thirdly, the prolongation of the cell cycle considerably enhanced the spatial regularity and clarity of microcolumn arrangement, suggesting that reduced proliferative dynamics provide a temporal buffer that enables more precise spatial coordination among developing neurons. This mechanism may represent a fundamental principle whereby developmental timing critically shapes the precision of neural circuit assembly. Our results emphasize that spatiotemporal coordination among progenitor cells is a key organizing principle in neocortical development. They also provide a theoretical framework linking dynamic cellular behavior to the emergence of higher-order cortical cytoarchitecture.

Disclosures: Y. Nariya: None. **H. Maruoka:** None. **S. Okabe:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.07/Web Only

Topic: A.01. Neurogenesis and Gliogenesis

Support: HRZZ-IP-2024-05-7157
HRZZ-IP-2022-10-5975

Title: Ultrastructural and molecular specification of laminar compartments in the medial pallium of the human fetal telencephalon during early mid-gestation

Authors: *I. KOSTOVIC[1], I. SALAMON[2], J. KOPIC[1], Z. KRSNIK[1];
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Abstract: The neurogenetic and histogenetic processes underlying the differentiation of allocortex within the medial pallium of the human embryonic brain are initiated around the 8 postconceptional week (PCW), a period when limbic cortex occupies a substantial portion of the medial aspect of the telencephalic vesicles. In this study, we examine the relationship between the compartmental distribution of proliferative, fibrillar, migratory, and synaptic markers with the laminar organization of synapses, as identified by electron microscopy (EM), within the mesocortical portion of the mid-cingulate cortex (MC). The analysis of the postmortem tissue of the human fetal brains aged 14-15 PCW revealed that high synaptic density per inspected area was observed within the enlarged marginal zone (MZ). Notably, synapses were also observed within the deep, not yet fully delaminated cortical plate (CP), along with a moderate number of synapses in the narrowing, extracellular matrix (ECM)-rich subplate. The distribution of molecular markers revealed the presence of thin outer subventricular zone (oSVZ; TBR2+), early joint separation of inner SVZ, and ventricular zone (SOX2+) by callosal periventricular fibers. The intermediate zone of the mesocortex is primarily composed of cingulum bundle fibers. The enlarged MZ consists of tangential fiber system, bifurcating apical dendrites originating from the CP cells, Reelin+ Cajal-Retzius cells, sparse proliferative cells, and ECM. In conclusion, the midgestational mesocortex represents a characteristic example of transitional cortical type derived from the medial pallium. It mirrors the archicortex in its predominant expression of superficial circuitry elements and narrowly developed oSVZ. However, it also shows resemblance to the structural pattern of the isocortex (neocortex) in terms of synaptic organization of CP. Lastly, the sustained presence of synapses in the deep, incompletely delaminated CP seems to be a hallmark of the mesocortex. Our findings confirm that early midgestation is a pivotal period for the establishment of basic synaptic organization within the limbic cortex and underscore the tectogenetic role of the callosal fibers. Although the mechanisms involved in specification of mesocortex remain poorly understood, they likely involve intrinsic genetic molecular mechanisms, interaction with massive interhemispheric mesenchymal structures, and sparse thalamic innervation. These normative data on the typical compartmental organization of the ventral midcingulate mesocortex should be taken into consideration in studies investigating the origin of neurodevelopmental disorders.

Disclosures: I. Kostovic: None. **I. Salamon:** None. **J. Kopic:** None. **Z. Krsnik:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.08/A7

Topic: A.01. Neurogenesis and Gliogenesis

Title: Assessing the progression of mitochondrial damage and dysfunction in Bak1 microexon knockout neurons leading to cell death

Authors: *Y.-L. LAM[1], L. LIN[1], S. ZHENG[2];
[1]Univ. of California, Riverside, Riverside, CA; [2]Univ. of California Riverside, Riverside, CA

Abstract: Genetic regulation of neuron longevity is poorly understood. Our previous studies demonstrate that intrinsic attenuation of neuronal apoptosis can be mediated through the neuron-specific, post-mitotic inclusion of *Bak1* microexon 5, resulting in a downregulation of BAK1 protein as neurons mature. BAK1 is a pore-forming protein, localized to the outer mitochondrial membrane that releases cytochrome C from the intermembrane space during the commitment step of apoptosis. Germline knockout of microexon 5 results in post-mitotic BAK1 induction, increased apoptosis in the brain, and perinatal lethality. To examine the longer term necessity of this regulatory mechanism, we have generated a conditional allele for neural specific deletion of exon 5 which results in BAK1 protein expression in a subset of post-mitotic neurons. Forebrain knockout leads to progressive, region-specific neuronal degeneration. With the goal of understanding the underlying mechanisms of this process, we examined how neuronal BAK1 induction resulting from microexon 5 deletion alters the behavior of mitochondria at various time points throughout the progression of neuronal loss. Biochemical, microscopic, and metabolic studies show signs of mitochondria dysfunction in our *Bak1* microexon 5 (Bak1-e5) conditional knockout mice, which express BAK1 protein post-mitotically. Early stages of neuronal loss are accompanied by electron transport chain-associated dysfunction rather than an accumulation of mitochondrial DNA damage, suggesting a potential mechanism that could link BAK1 induction to neuronal loss through mitochondria. Studying the post mitotic induction of BAK1 on mitochondria dynamics and function has potential implications in understanding how neurons maintain their unique properties of energetic metabolism and lifespan.

Disclosures: Y. Lam: None. **L. Lin:** None. **S. Zheng:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.09/Web Only

Topic: A.01. Neurogenesis and Gliogenesis

Support: INPer_2018-1-146

Title: Effect of maternal hyperglycemia on cortical neuronal migration: hypofunction of reelin signaling

Authors: *R. VALLE-BAUTISTA[1], I. SOTO VILLANUEVA[2], D. A. DÍAZ PIÑA[2], V. CHÁVEZ PÉREZ[2], E. IDIAQUEZ HERNÁNDEZ[2], N. E. DIAZ[3], A. MOLINA[4];
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Abstract: The cerebral cortex is a laminar structure derived from the dorsal telencephalon. In rats, corticogenesis begins on embryonic day 10 (E10) with the expansion of neural stem cells, followed by neurogenesis and the onset of neuronal migration at E12. Migration begins with the displacement of neuroblasts along the radial glia (RG) scaffold, and upon sensing REELIN (RLN), they stop to settle in the appropriate layer. This process gives rise to the characteristic inside-out pattern of cortical development. Without RLN, this pattern is inverted, resulting in cognitive and motor impairments. We had reported that 21-day-old (P21) pups from diabetic rats exhibit disorganization of the motor cortex layers and increased expression of neuronal markers for layers II-VI, including SATB2, FOXP2, and TBR1. Additionally, pyramidal neurons in layers V-VI exhibit reduced excitability and dendritic arborization compared to controls. This study aimed to determine changes in the RLN signaling pathway's activation in rat embryos' cortical neuroepithelium. To this aim, we evaluated several components of the RLN pathway, such as RNL itself, the activation of DAB1, ApoER2, VLDLR, and N-CADHERIN (N-CAD) at E16, while in neonates and P21 pups, the cortical cytoarchitecture was evaluated. The results show that maternal hyperglycemia decreases the expression of RLN, VLDLR, and P-DAB1 at E16, reduces the interaction between neuroblasts and the RG scaffold regulated by N-CAD, and increases the migration of pyramidal neurons expressing CUX1 and SATB2, promoting the invasion of layer I at P21. Our results suggest that embryos from diabetic dams present a hypofunction RLN, probably through VLDLR promoting aberrant neuronal migration through RG that further contributes to altered neuronal polarity and postnatal cortical stratification.

Disclosures: R. Valle-Bautista: None. **I. Soto Villanueva:** None. **D.A. Díaz Piña:** None. **V. Chávez Pérez:** None. **E. Idiaquez Hernández:** None. **N.E. Diaz:** None. **A. Molina:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.10/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01AA023508,
SRS Undergraduate research program

Title: Bioinformatic Analyses of Cell Death Genes in the Developing Neocortex of Mice: Evaluation of Genetic Regulation and Gene Networks

Authors: W. PERRIN[1], L. LU[1], ***K. M. HAMRE**[2];
[1]Univ. of TN Hlth. Sci. Ctr., Memphis, TN; [2]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Cell death is a critical process during brain development, specifically in the elimination of surplus neurons. However, as the brain matures, the importance of cell death processes decreases, and by adulthood, it transitions from a normal developmental process to a pathological one triggered by insult or injury. Cell death is a global term encompassing several types and pathways. In the present study, cell death was examined in the developing and adult neocortex. Expression of cell death genes, across multiple pathways, was examined using a genetic reference population, BXD mice, at several timepoints to assess 1) the degree to which the genetic regulation of expression of these genes changes over time and 2) whether the downstream pathways related to these genes also change over time. This research focused on expression during postnatal days (P) P3, P14, and adulthood (>P60), which have differing levels of apoptosis and developmental characteristics. The earliest timepoints encompass the brain growth spurt, which is heavily characterized by synaptogenesis. Gene expression databases from these ages in the neocortex of BXD mice were analyzed. Bioinformatic analysis, utilizing GeneNetwork.org and Gene Ontology, was used to compare upstream expression quantitative trait loci (eQTLs), and correlations were assessed to evaluate changes in expression in apoptotic genes and their networks, along with revealing biological processes, and phenotypes that are similar and different across time. From the initial pool of cell death genes, 17 genes were found to have similar eQTLs across two or more of the three maturation periods, suggesting similar regulation across time. These 17 genes were searched for correlating genes in each of the three developmental stages, with P3 containing 1,045 correlated genes, P14 containing 839 correlated genes, and adults with 394 correlated genes, all of which were used to define the downstream network. Correlational analyses identified several processes that spanned across all of these stages, including metabolic processes, signaling receptor activity, and synapses. Additionally, 11 phenotypes, including drug response, locomotor activity, and dopamine levels, correlated with the gene networks linked to the original 17 genes across maturation stages. These results provide evidence that while the importance of cell death pathways diminishes over time, the regulation of gene expression as well as associated genetic pathways remains largely consistent through later neocortex maturation.

Disclosures: W. Perrin: None. **L. Lu:** None. **K.M. Hamre:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.11/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01NS1399142
NSF 2235566

Title: Crk adaptor proteins regulate cortical lamination

Authors: *P. A. RAO[1], A. M. TREPTOW[2], M. M. RICCOMAGNO[3];
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Abstract: Proper neuronal migration during cortical lamination is essential for functional circuit development. Disruptions in this process lead to a host of neurodevelopmental disorders hallmarked by an array of functional deficits. Cortical migration requires Reelin signaling. Reelin is secreted in the marginal zone by Cajal-Retzius cells and binds to its receptors in migrating neurons, leading to phosphorylation of the adaptor protein Disabled-1 (Dab1). Dab1 subsequently activates downstream signaling to regulate cell adhesion and cytoskeletal remodeling in migrating cortical neurons. However, the molecular mechanisms linking Dab1 activation to cytoskeletal remodeling and migration remain poorly understood. The chicken tumor virus #10 regulator of kinase, or Crk, family of adaptor proteins (Crk and CrkL) are well-positioned to bridge Reelin signaling to actin and adhesion dynamics via interactions with integrin adhesion complex (IAC) components and other cytoskeletal regulators. Here, we investigated the role of Crk and CrkL in cortical lamination using conditional gene knockout strategies in embryonic mouse cortex. We established a *Crk and CrkL double conditional knockout (dcKO)* model and assessed effects on neuronal positioning using layer-specific markers at postnatal day 7 (P7). Loss of both Crk and CrkL led to severe cortical layering defects resembling those observed in *Reelin* or *Dab1* mutants, with cortical layers appearing inverted: in mutants, superficial layer markers are present closer to the ventricular zone while deep layer markers are present close to the pial surface, opposite of what is seen in control animals. These results demonstrate that Crk and CrkL are required for proper cortical lamination, and may act downstream of Reelin-Dab1 signaling, coordinating neuronal positioning during cortical development. Future work will focus on molecular epistasis analysis to determine whether Crk and CrkL indeed act downstream of Reelin and testing the functional role of Crk proteins in cell adhesion and migration.

Disclosures: P.A. Rao: None. **A.M. Treptow:** None. **M.M. Riccomagno:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.12/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: Program Development Grant - University of Massachusetts Boston

Title: Sex specific androgen neuroprotection in the developing hippocampus

Authors: *E. K. PRITCHARD, S. L. ZUP;
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Abstract: Early testosterone exposure can modulate naturally occurring programmed cell death, or apoptosis, during neurodevelopment. Differential apoptosis controlled by gonadal hormone exposure is responsible for creating sex differences in the volume of some brain regions. The brain's learning and memory center, the hippocampus, is often reported to be slightly larger in males, but it is unknown if testosterone action shapes this sex difference. Previous work from our lab found that at day in vitro (DIV) 7, male hippocampal cultures have significantly higher levels of apoptosis after mild glutamate stimulus than female cultures, and pretreatment with dihydrotestosterone (DHT) protects against apoptosis for male neurons. At the end of the first week of life (DIV7), the excitation-inhibition (E/I) balance is especially volatile, with GABA ceding its role as the primary excitatory neurotransmitter to glutamate. This E/I balance continues to be fine-tuned, leaving neurons vulnerable to excitotoxicity, into the second week of postnatal development. l. We investigated whether androgen neuroprotection in the hippocampus persists into the second week. On the day of birth, rat hippocampi were dissected and used for sex specific primary neuronal culture. Cultures were either treated with 1nM DHT or dimethyl sulfoxide on DIV 0 and 1, and a series of 3 10uM glutamate treatments began on DIV 9. On DIV 10, the third glutamate treatment was administered, and cultures were fixed for the TUNEL assay 4 hours later, in order to quantify apoptosis. A 3-way ANOVA was conducted with the factors of sex, DHT, and glutamate treatment (total n=60 coverslips; n=7-8 per condition). Post hoc, we utilized simple effects testing to clarify significant interaction effects. Our data demonstrated that males had significantly higher levels of glutamate-induced apoptosis compared to females, and that DHT pretreatment significantly decreased glutamate induced apoptosis regardless of sex (all $p \leq 0.01$), with a more dramatic decrease seen in males. This work confirms the pattern of apoptosis and androgen neuroprotection seen in DIV7, and we will further explore the mechanisms underlying the androgen neuroprotection in future studies. Characterizing sex specific hippocampal development will expand our understanding of why many neurodevelopmental disorders are sexually dimorphic in prevalence and clinical presentations.

Disclosures: E.K. Pritchard: None. **S.L. Zup:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.13/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: National Science and Technology Council (NSTC 113-2923-E-038-001; NSTC 114 -2927-I-038 -504)
National Health Research Institutes (NHRI-EX114-11431NI) of Taiwan

Title: Human Platelet Lysate Enhances Neural Stem cell Activity in SVZ and Stimulates Local Neurogenesis in OB of Young Adult Mice

Authors: *A. SRINIVASAN[1], A. NYAMERDENE[1], D. BLUM[3,4], T. BURNOUF[1,2];
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Abstract: Adult neurogenesis in the adult brain is, in part, localized to the subventricular zone (SVZ), with neural progenitors migrating through the rostral migratory stream (RMS) to the olfactory bulb (OB). Human Platelet Lysate (HPPL), which constitutes the platelet secretome, is a biologically active concentrate rich in multiple trophic factors and extracellular vesicles, was investigated for its ability to modulate adult neurogenesis in the SVZ and olfactory bulb of 8-week-old mice. HPPL was prepared from platelets isolated from clinical-grade platelet concentrates obtained from healthy donors and characterized by measuring total protein and growth factor content, and by LC-MS/MS proteomics. Ex vivo experiments using SVZ- derived tissues demonstrated a significantly higher neurosphere number and diameter under 0.5% (v/v) HPPL treatment (n=5). These SVZ findings were further supported by in vivo experiments, where three-day intranasal administration of 30 µL HPPL significantly increased neural progenitor proliferation in the SVZ, as evaluated using EdU labeling. We next investigated whether HPPL could also act directly on OB resident progenitor cells, independently of SVZ input. OB tissues were isolated from wild-type adult mice, and primary cultures were established under neurosphere-forming conditions. Neurospheres were obtained, their number remaining consistent from P0 to P2 in serum-free medium composed of Neurobasal medium supplemented with B27, EGF, bFGF, and GlutaMAX, averaging 226 ± 26 neurospheres per 96-well plate. These findings support a self-renewal capacity of OB progenitor cells independent of SVZ contribution. A set of OB-derived tissues culture was then treated with 0.1 to 2.0 % HPPL. 0.5% (v/v) HPPL yielded the highest neurosphere count, while 1.0% (v/v) resulted in the largest average sphere diameter, both being significantly increased compared to the untreated control group . These findings suggest that HPPL supports stem cell proliferation not only in the SVZ niche but also in OB. In vivo long-term maturation experiments at D28 EdU post-administration demonstrated a higher numbers of EdU+ cells and EdU+/NeuN+ neurons in the OB granule cell layer (GCL), supporting that HPPL treatment enhances the neuronal fate of stem cells in this region. Further investigations are needed to confirm the mechanisms underlying the ability of HPPL to induce neurogenesis in the OB and to distinguish between direct local effects in OB resident stem cells and their RMS mediated migration from the SVZ.

Disclosures: A. Srinivasan: None. **A. NyamErdene:** None. **D. Blum:** None. **T. Burnouf:** Other; Co-inventor of patents owned by TMU and ULille-Inserm; co-funder of Invenis Biotherapies..

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.14/A12

Topic: A.01. Neurogenesis and Gliogenesis

Support: Grant from Else Kröner-Fresenius-Stiftung to Sonja Bröer (2022 EKEA.132)

Title: Seizure-induced cell fate shifts: Tracking neurogenesis in a viral encephalitis-induced epilepsy model

Authors: *E. WEISS[1,2], A. PAULETTI[1], A. EGILMEZ[1,2], S. C. BRÖER[1,2];
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Abstract: Neurogenesis, the generation of new neurons, is noted to be disrupted in conventional rodent models of epilepsy, where aberrant maturation and migration of neural stem cells (NSC) contribute to circuit hyperexcitability and cognitive dysfunction. Accelerated proliferation of neural progenitors is described as one main difference after initial insult, which adds to a changed network connectivity. Despite central nervous system (CNS) infections being a leading cause of seizures, the impact of encephalitis-associated seizures on NSC dynamics and differentiation remains largely unexplored. Our research seeks to characterize neurogenesis and define critical therapeutic windows during which modulation of neurogenesis may alter the course of epileptogenesis following CNS infection. By intracortically infecting male C57Bl6/J mice at the age of 7-8 weeks with the Daniel's strain of Theiler's Murine Encephalomyelitis Virus, we induced acute seizures in 30-45 % of infected mice. A subset of mice also develops chronic epilepsy several weeks after infection. Mice were sacrificed at 7 (n=28), 14 (n=16), 28 (n=14) and 90 (n=58) days post infection (dpi) to assess neurogenic responses in the subgranular layer of the hippocampal dentate gyrus. A combination of immunohistochemical markers together with *in vivo* injected BrdU as a proliferation marker were used to follow cell fates. Reactive gliosis, including proliferation of microglia and astrocytes, was elevated in a time-dependent manner post-infection. In contrast, we observed a significant reduction in doublecortin-positive (DCX^+) immature neurons at 7 and 14 dpi, with more severe depletion in mice that experienced seizures. Surviving DCX^+ cells showed ectopic migration. While neural progenitor proliferation was obliterated in the acute phase (7 dpi) in seizing animals, the number of mature neurons ($BrdU^+/NeuN^+$) born during the acute phase was unaffected at 28 dpi. This could indicate an activated and accelerated proliferation after acute depletion as seen in other models of epilepsy. Ongoing BrdU-based fate mapping at chronic timepoints will determine the long-term development of NSC dynamics. Together, these findings suggest that seizure activity following viral CNS infection significantly alters neurogenic processes, potentially shifting NSC fate and contributing to epileptogenesis.

Disclosures: E. Weiss: None. **A. Pauletti:** None. **A. Egilmez:** None. **S.C. Bröer:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.15/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS110586

Title: Eph-ephrin signaling in the developing mouse rostral migratory stream

Authors: *C. DE SILVA[1], D. YEROSHENKO[1], A. BURLI[2], J. C. CONOVER[3];
[1]Dept. of Physiol. and Neurobio., [2]Dept. of Mol. and Cell. Biol., [3]Dept. of Physiol. & Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: Molecular mechanisms guiding controlled, long-range migration of newly generated neuroblasts from the ventricular-subventricular zone (V-SVZ) along the rostral migratory stream (RMS) to the olfactory bulb (OB) are poorly understood. We present developmental progression of this pathway in mouse from P6-P60. Specifically, we show that at P6 there is a loose amalgamation of migratory neuroblasts. This develops into a compact, fasciculated array of neuroblasts and associated astrocytes by P12, which by P60 then progresses to a very dense, albeit reduced, bundled array of neuroblasts that migrate through a meshwork of astrocytes. Previous work in our lab showed that the receptor tyrosine kinase EphA4 is crucial for maintenance of the compact, adult RMS organization and directed migration of neuroblasts to the olfactory bulb. Since other Eph receptors and their cognate ligands, the ephrins, are expressed in the RMS by neuroblasts and astrocytes throughout development, we sought to discern how Eph-ephrin signalling mediates regulated migration at P6, P12 and P60. First, we characterized transcriptional profiles and then we validated these profiles by examining Eph-ephrin signalling based on phosphorylation patterns. Eph receptors, 9 EphAs and 5 EphBs, bind to ephrinA/B ligands and have the capacity for bi-directional signalling, adding complexity to Eph-ephrin signalling mechanisms. Using single-cell RNA sequencing and high-resolution clustering of RMS cells, we show that RMS neuroblasts subcluster into 3 groups based on unique Eph and ephrin transcriptional RNA profiles, while all RMS astrocytes show similar Eph/ephrin profiles. These profiles remain the same from P6, P12 and P60; however, the cell composition of the RMS changes. Using CellChat, we modelled the probabilities of specific cell-cell interactions based on Eph and ephrin expression. This distinguished both neuroblast-neuroblast and neuroblast-astrocyte communications mediated through Eph-ephrin combinations. To validate these combinations, we identified activated, phosphorylated forms of Eph and ephrins in RMS neuroblasts and astrocytes. This allowed us to narrow Eph-ephrin signalling to specific receptor-ligand interactions. Specifically, we show that at P6 EphB1/2 and ephrinB1/2/3 mediated signalling is prominent. By P12, EphA3/4/5 signalling increased significantly, and increased

further by P60. Our findings suggests that as the RMS constricts and cell composition changes from P6 to P60, Eph-ephrin signalling also changes. Identifying the molecular mechanisms guiding postnatal neuroblast migration will advance our understanding of neurodevelopmental disorders.

Disclosures: C. de Silva: None. **D. Yeroshenko:** None. **A. Burli:** None. **J.C. Conover:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.16/A14

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH IRP Grant ZIAMH002898
NIH Grant P50HD103536

Title: Aav-mediated gene transfer to developmentally timed neuronal cohorts in the primate brain

Authors: *A. RIBEIRO GOMES[1], N. WRIGHT[1], S. S. MASTWAL[1], L. BAWEKE[1], C. T. RICHIE[3], T. B. USDIN[2], K. H. WANG[4], D. A. LEOPOLD[1];
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Abstract: Understanding how early developmental events unfold to shape human brain organization has been hampered by limited genetic access to neurodevelopmental processes in strategic animal models, most notably primates. To overcome this bottleneck, we recently developed a fast, flexible and scalable method for widespread neuronal transduction in marmosets using ultrasound-guided fetal intracerebroventricular injections (FIVI) of recombinant adeno-associated virus (rAAV) (Ribeiro Gomes et al., 2025). Using FIVI, we showed that fetal rAAV injections enable transgene expression in distinct neuronal populations, which are specified based on the gestational timing of injection and, unexpectedly, the rAAV serotype. Notably, certain rAAV serotypes transduced cortical neurons in a manner that was time locked to neurogenesis, with earlier injections primarily labeling deeper-layer neurons and later injections targeting more superficial layers. To test the specific connection to neuronal birth timing, we administered rAAV via FIVI at the same time as an intraperitoneal administration of the thymidine analogue 5-ethynyl-2'-deoxyuridine (EdU), as the EdU is known to label progenitors dividing within a narrow time window. This combined approach thus allows for the tagging of neurons born within approximately two hours of rAAV exposure and enables assessment of how the viral transduction aligns with neuronal birthdate. In rats, pilot experiments

using the same approach indicated that rAAV2 exhibited the strongest correspondence with EdU-labeled cells, prompting us to focus on this serotype in our study. In marmosets, preliminary results reveal strong spatial overlap between EdU-labeled populations and rAAV2-transduced neurons, suggesting that, as in rats, rAAV2 preferentially targets neurons generated near the time of FIVI injection and could serve as a tool for time-specific gene delivery during neurogenesis. We are currently evaluating whether the spatial distribution of EdU-labeled and rAAV2-transduced populations aligns across brain regions and FIVI gestational timepoints, as well as characterizing the specific cell types targeted. This work will help develop strategies to deliver genetic tools to map and manipulate developmentally defined cells, thereby opening new opportunities to bridge developmental trajectories with brain function across the primate's lifespan.

Disclosures: A. Ribeiro Gomes: None. **N. Wright:** None. **T.B. Usdin:** None. **K.H. Wang:** None. **D.A. Leopold:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.17/A15

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 5F31NS118982

Title: Micrornas mediate spatiotemporal control of cortical interneuron migration and specification

Authors: *J. X. DU[1], N. ZOLBOOT[2], F. ZAMPA[2], G. LIPPI[2];
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Abstract: The development of cortical inhibitory interneurons (cINs) has been fine-tuned to produce a highly specialized population that serves as the substrate for cortical circuit assembly. Understanding the developmental programs responsible for the precise distribution and diversification of cINs is key to unlocking the principles of building and evolving brain circuits, yet much remains unknown about their key regulators and mechanisms. MicroRNAs (miRNAs) are a class of non-coding RNAs that are necessary for cIN development; genetic knockout (KO) of miRNAs in developing cINs causes severe defects in their abundance, migration, and expression of the mature markers parvalbumin (PV) and somatostatin (SST). However, due to the coarse temporal resolution of global miRNA knockout experiments, it remains unclear how and when miRNAs directly regulate cIN specification and migration. Further, little is known about miRNA-mediated mechanisms of cIN development, in part because the miRNA-target network specific to developing cINs is poorly understood.
We engineered an innovative miRNA toolbox with vastly improved temporal resolution and

molecular specificity in order to unlock mechanistic insight on miRNA regulation of cIN development. Single cell transcriptomics showed that defects induced by miRNA KO in cINs, including developmental delay and altered cell type specification, are already evident during the embryonic period. By inducing miRNA KO only during late embryonic development, we revealed an irreversible decrease in the ratio of PV:SST cINs, likely through alterations in programmed cell death of these two classes. To identify specific miRNA-mediated mechanisms of cIN development, we profiled miRNA-target interactions (mTIs) in cINs during neurogenesis and migration, overcoming the technical challenges of targetome profiling in low abundance cell types. We performed an unbiased analysis of gene-level miRNA load, identified putative nodes in the miRNA-target network termed "miRNA hotspots", and screened the top miRNA hotspots for functions in cIN migration. Dysregulation of the miRNA hotspot Ist1 decreased migration speed, increased neurite branching, impaired dispersion across cortical regions, and promoted premature cortical invasion. We further discovered a novel mechanism by which miR-125b preferentially represses Ist1 within the migratory streams, creating spatiotemporal patterning of Ist1 expression that delineates phases of cIN migration. Our experimental framework is able to uncover novel molecular mechanisms, down to the functions of individual mTIs, in a range of developmental processes key to circuit assembly.

Disclosures: J.X. Du: None. **N. Zolboot:** None. **F. Zampa:** None. **G. Lippi:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.18/A16

Topic: A.01. Neurogenesis and Gliogenesis

Support: University Research Board at American University of Beirut (AUB)
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Kamal Shair Central Research Science Laboratory (KAS CRSL)

Title: Rb substantially compensates for the double loss of p130 and p107 in adult but not embryonic neural stem cell lineages

Authors: *R. Y. SWAIDAN[1], A. DAHER[1], A. BEJJANI[2], Y. E. EL ATIE[1], Y. CHEHAB[1], R. BOU HAMDAN[1], R. VANDENBOSCH[3], R. S. SLACK[4], S. OMAIS[5], N. GHANEM[1];
[1]American Univ. of Beirut, Beirut, Lebanon; [2]Cincinnati Children's Hosp. Med. Ctr., Ohio, OH; [3]Univ. of Liège, Liège, Belgium; [4]Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; [5]Natural Sci., Lebanese American Univ., Beirut, Lebanon

Abstract: The Retinoblastoma (Rb) family of pocket proteins (p107, Rb, and p130) regulates all stages of neurogenesis from stem cell activation to maintenance of long-term neuronal survival in the brain. Earlier studies have demonstrated non-overlapping, often complementary, functions for these cell cycle regulators with potential functional compensation. However, it is still not clear to what extent compensation and/or synergy exist between family members during neural stem cell (NSC) lineage development. Fong et al. recently reported that a triple knock-out (TKO) of all pocket proteins driven by Nestin-Cre leads to a transcriptomic switch from NSCs' quiescence to activation, followed by niche exhaustion in the adult hippocampus. Here, we used the same Nestin-CreERT2-YFP inducible lines to investigate whether pocket proteins are equally critical in NSCs fate regulation in the adult subventricular zone (aSVZ) and during embryogenesis. We generated young adult mice carrying, in combination, double or triple deletions of the Rb protein family, as well as triple heterozygous control mice. During development, we generated triple-knockout, double-knockout, and Rb-knockout embryos and conducted phenotypic analyses in comparison to WT embryos at E14.5. Statistical analyses were performed using independent two-tailed Student's T-tests and a minimum of three animals per genotype for each experiment. We report that loss of all pocket proteins in the aSVZ leads to fast NSCs activation, ectopic progenitor proliferation, and massive apoptosis, all of which cause niche depletion and premature loss of neurogenesis inside the olfactory bulb (OB). Importantly, a p107-p130 double knockout carrying a single wild-type Rb allele (DKO) significantly rescues the above defects and preserves adult neurogenesis. On the other hand, TKO embryos display significant disruptions during all stages of neurogenesis at E14.5, resulting in embryonic lethality. Analogous defects are observed upon loss of any five out of the six alleles of pocket proteins, with only partial rescue of the proliferation defects noted in DKO embryos carrying one wild-type Rb allele. The above TKO phenotypes are partly mediated by opposite deregulations in the Notch-Hes signaling pathway in the embryonic versus the adult brain, which are in turn linked to opposite variations in E2F3a and E2F3b embryonic gene expressions. Our data prove that Rb is an essential pocket protein in the control and maintenance of adult OB neurogenesis and, interestingly, reveal the existence of interchangeable, dose-dependent functions of pocket proteins in controlling neuronal differentiation and survival during development.

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Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.19/A17

Topic: A.01. Neurogenesis and Gliogenesis

Support: DBT- DBT-Har Gobind Khorana Innovative Young Biotechnologist Fellowship (BT/13/ IYBA/2020/02)

EMBO Global investigator (#6080)
DBT/Wellcome Trust India Alliance: Intermediate Career Fellowship (IA/I/19/1/504288)

Title: Human brain enriched extra cellular matrix genes regulate cortical folding and development

Authors: *A. CHANNAKKAR;
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Abstract: Cortical folding and expansion are hallmark features of primate brain evolution and development, critical for optimizing functional organization, and neuronal connectivity. Disruptions in these processes are linked to neurodevelopmental disorders such as lissencephaly and epilepsy. The extracellular matrix (ECM) is a key regulator of cerebral cortex development, influencing radial glial cell (RGC) proliferation, basal progenitor (BP) amplification, and tissue biomechanics. In this study, we demonstrate that LSD1, a histone demethylase regulating neural stem cell (NSC) neurogenesis, modulates ECM gene expression. Inhibition of LSD1 in human NSCs upregulated a subset of ECM genes with human brain-enriched expression, particularly in the subventricular zone (SVZ), a germinal layer pivotal for cortical expansion. Strikingly, overexpression of these ECM genes in mouse embryonic cortex (E13.5) via in utero electroporation induced cortical fold-like structures. This folding coincided with cell and non-cell autonomous delamination of BPs, as well as APs (apical progenitors) suggesting ECM-mediated alterations in tissue stiffness and progenitor dynamics. Comparative epigenomic analysis revealed human-specific enhancer signatures (H3K4me2/H3K27ac) at loci encoding ECM and cell adhesion molecules, implicating evolutionary adaptations in cortical folding mechanisms. Our study highlights a human-specific epigenetic mechanism that regulates the expression of human brain-enriched extracellular matrix (ECM) genes, which in turn modulate key neurodevelopmental processes unique to the human brain.

Disclosures: A. Channakkar: None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.20/A18

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS116054

Title: Cellular context matters: Use of a genetic mosaic mouse model to identify autonomous and non-autonomous effects of cytokinesis gene mutations in the developing cortex

Authors: *H. DINGSDALE[1], E. ROSS[1], S. SHETTY[1], K. FILIPEK[1], N. DWYER[2];
[1]Cell Biol., [2]Cell Biol, Univ. of Virginia, Charlottesville, VA

Abstract: Correct cortical development relies on tight control of neural stem cell (NSC) division and differentiation. Mitosis and cytokinesis are precisely timed and strictly oriented relative to the ventricular surface to allow accurate cell separation and segregation of fate determinants. The last stage of division, abscission, plays a key role in daughter cell fate and function. Disrupting the timing or plane of abscission frequently leads to early cell cycle exit or cell death, with drastic effects on cortical development. Our previous data identified two abscission genes that play key roles in cortical development, *Cep55* and the kinesin, *Kif20b*. Loss of these genes leads to delayed or failed NSC divisions, elevated apoptosis, and microcephaly; cortical layering however is undisturbed. The extensive cell death, abnormal architecture, and effects on non-neural knockout cells make it challenging to distinguish between cell-autonomous effects and those arising from the disrupted microenvironment. To isolate the direct effects of these abscission genes in NSCs, here we created genetically mosaic mice, using Mosaic Analysis of Double Markers (MADM) to generate cortex-specific, fluorescently-labelled sparse knockout cells within an otherwise normal size brain. Using Emx1-Cre to drive MADM mosaicism in NSCs, we show that cells lacking *Cep55* or *Kif20b* have altered survival compared to their wildtype counterparts at postnatal day 0, even when these cells exist in identical environments. In MADM-*Cep55* mosaic brains, wildtype cells outnumber knockout cells at a ratio of 4:1. Later born cells in upper layers of the cortex appear more vulnerable to these effects, suggesting that cumulative damage across generations of NSCs may be contributing to cell loss. In addition, previous flow cytometry analysis of full *Cep55* knockout mice identified a large increase in the number of binucleate cells present in the developing cortex, as daughter cells are unable to complete the final stages of abscission. Leveraging the mosaic fluorescence generated by the MADM technique, we identified binucleates in situ in MADM-*Cep55* mice. Whilst 10% of knockout cells in a MADM-full *Cep55* knockout environment were binucleate, only 5% of knockout cells were binucleate in the genetically mosaic cortex, i.e. when neighboring cells were phenotypically normal. This suggests that non-cell-autonomous factors from the surrounding environment exacerbate the issues with cytokinesis. These findings shed new light on the complex network of interactions driving cortical development, helping clarify mechanisms driving microcephaly and other cortical malformations.

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Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.21/A19

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH MH113257

Title: Artificial intelligence-powered analysis of ependymal cell development in the macaque choroid plexus

Authors: *E. BURKE[1,2], M. RODOV[2], L. GREENE[2], P. RAKIC[2], A. DUQUE[2];
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Abstract: Archived materials in Collection 1 of the MacBrain Resource Center (MBRC) are being used in a *de novo* study to track the birth and distribution of ependymal cells in the choroid plexus (cp). The materials include rhesus macaque brains (NHPs) labeled with tritiated thymidine (^{3}H-TdR injection) and processed via autoradiography by Dr. Pasko Rakic in the 1970s. Using modern imaging and computational tools, we are studying cp development across embryonic and early postnatal stages in the lateral, third, and fourth ventricles - without the need to sacrifice new animals. This promotes cost-effective NHP research, reduces animal use, and improves our understanding of cp development and its role in CSF regulation, neuroinflammation, and other conditions. In ~ 30 NHPs, coronal sections near the mid rostro-caudal axis are being compared. Analysis began with light microscopy to find the earliest birth-dated (i.e., silver labeled) cp ependymal cells in the lateral ventricles. Next, sections were compared toward at least one of the following aims: First, to pinpoint the timeline of cp cell genesis (using embryos injected/sacrificed on the same day, e.g., E45/E45). Second, to determine location of cells born on different days at ~ P60 (e.g., specimen injected/sacrificed E45/P58 or E90/P63). Third, to trace cell migration from a single birth date through development (all monkeys injected on the same day, but sacrificed after different survival periods, e.g., E45/E59, E45/E78). After initial review, select sections were scanned at 20x magnification in an Aperio CS2 HR scanner (Leica) for image analysis and quantification. Preliminary findings show ependymal cell labeling as early as E36 in the lateral ventricle cp, with high labeling density through E90. Labeling begins to decline between E90 - E110 and continues decreasing through birth. Some labeled cp cells were observed after postnatal ^{3}H-TdR injections, aligning with rodent and human data supporting cp development beyond birth. Patterns are emerging in labeled cell locations within the cp, depending on age at injection/sacrifice and in relation to the mid-wall attachment point, but this warrants further analysis. Current efforts involve applying artificial intelligence (AI) -based image analysis tools (Aiforia) to supplement observational results and quantify total vs. labeled ependymal cells across ages. Given counts can exceed 10,000 cells per section of cp, automated tools may prove crucial for scaling up analysis. Future plans include analyzing more rostral and caudal coronal sections to strengthen 3D proliferation tracking and begin comparisons in other ventricles.

Disclosures: E. Burke: None. **M. Rodov:** None. **L. Greene:** None. **P. Rakic:** None. **A. Duque:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.22/A20

Topic: A.01. Neurogenesis and Gliogenesis

Support: Scholarship Akademie für Tiergesundheit e.V.
Grant START of Freie Universität

Title: Astrocyte Proliferation and Morphology Across Disease Progression in Viral Encephalitis-Induced Epilepsy

Authors: *A. EGILMEZ[1,2], E. WEISS[1,2], A. PAULETTI[1], S. C. BRÖER[1,2];
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Abstract: Viral encephalitis represents a major cause of infection-induced seizures and subsequent epileptogenesis. Astrocytes are central players in CNS inflammation and undergo functional alterations after seizures, such as morphological changes, and increase in numbers. This reactive astrogliosis may result from proliferation of existing astrocytes or seizure-induced switches in neural stem cell fate. This phenomenon has been demonstrated robustly in chemical epilepsy models, however there is no data on infection-induced seizures. This study characterizes astrocyte proliferation and morphological changes across disease phases using the Theiler's Murine Encephalomyelitis Virus (TMEV) model. Adult C57BL/6J mice were infected intracerebrally, and BrdU was administered during the following acute phase of encephalitis to label proliferating cells. Seizures were monitored via EEG and behavioral observations. Mice were sacrificed at various time points post-infection. TMEV-infected animals demonstrated increased astrocyte reactivity in the acute phase of encephalitis, and astrocyte density was positively correlated with seizure activity. Proliferation of radial glia was reduced, and proliferation of mature astrocytes was increased. Morphological assessment showed regional variation but no between-group differences except larger astrocytes in seizure animals' molecular layer. This study reveals temporal astrocyte proliferation and morphological changes during viral encephalitis-induced seizures. Ongoing studies aim to characterize complete temporal profiles (28 and 90 dpi) to identify the mechanisms underlying the transition from acute inflammation to chronic epilepsy.

Disclosures: A. Egilmez: None. **E. Weiss:** None. **A. Pauletti:** None. **S.C. Bröer:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.23/A21

Topic: A.01. Neurogenesis and Gliogenesis

Support: Oscar Gregory Endowment in Stroke and Brain Research
Chan Zuckerberg Biohub
NIH Grant 1DP2NS122550-01
Rita Levi Montalcini scholarship from the Dompé Foundation

Title: Regional specialization and progenitor dynamics in the caudal ganglionic eminence

Authors: *E. HORTON[1], M. F. PAREDES[2];
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Abstract: The prenatal brain generates inhibitory interneurons in a region called the ganglionic eminence (GE), which can be subdivided into the medial, lateral, and caudal GEs (MGE, LGE, and CGE respectively). Each GE subregion produces a distinct set of neuronal subtypes. The CGE produces a wide variety of interneurons that migrate to different regions including the cortex, hippocampus, and amygdala. How the diversity of CGE derived interneurons is established in the mammalian cortex is unknown. To understand their origins, we investigated the transcriptional diversity among progenitors within the human CGE (hCGE). We identify the (hCGE) at 23 GW (gestational weeks) by the expression of transcription factors NR2F2 and PROX1 and its relationship to anatomical landmarks, such as the thalamus and hippocampus. Whole genome spatial transcriptomics on the hCGE at 23 GW identified a compartment at the ventricular wall with high Vimentin and FABP7 gene expression, confirming the localized presence of radial glia-like progenitors within a ventricular zone (VZ). There were fewer proliferating cells in the VZ compared to the adjacent region removed from the wall, which we termed the inner VZ (iVZ). This suggests most of the proliferation occurs outside of radial glia progenitors, within cellular populations at the iVZ of the hCGE. Gene expression patterns differed across the dorsal and ventral portions of the hCGE. When compared to the dorsal hCGE, the ventral hCGE was highly proliferative and enriched for EGFR as well as many histone modifiers, pointing to the dynamic nature of this region. Our data supports spatial heterogeneity of hCGE progenitors at 23GW. This research will help us understand the origin of CGE neuronal subtypes and elucidate the mechanisms at play in CGE progenitors available to affect the fates of their progeny. Additionally, comparing hCGE features to that of the CGE in rodents and primates can help identify human-enriched features of brain development and the molecular mechanisms that underlie those comparative differences.

Disclosures: E. Horton: None. **M.F. Paredes:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.24/A22

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF GFRP
ARC Fellowship
The Brain Research Foundation Seed Grant
The Shurl and Kay Curci Foundation
The Gabilan Faculty Award
NIMH 1R01MH136258

Title: Optimized floating cortical explant culture systems to support embryonic neurogenesis

Authors: *B. ZHOU[1], R. M. FAME[2];
[1]Neurosciences Interdepartmental Program, [2]Neurosurg., Stanford Univ., Stanford, CA

Abstract: Cerebrospinal fluid (CSF), which is generated by the choroid plexus (ChP) in adults, first arises from amniotic fluid (AF) after neural tube closure. CSF is physically separated from AF and contains distinct molecular and ionic components to support the neurogenic niche during embryonic cortical development (Fame, et al. 2020). However, the relationship between CSF composition and neural progenitor development remains poorly understood. As CSF directly interacts with cortical progenitors along the apical ventricular surface, it is poised to support corticogenesis and neural maturation. Many CSF solutes change over early development including metabolites, neurotransmitters, and ions (Xu*, Fame*, et al 2019; Fame*, Fame, et al. 2024, Hedner, et al. 1982). Floating cortical explant culture on artificial CSF (aCSF) allows for direct modulation of early brain environment and has proven useful to test how individual CSF solutes can support neural progenitors (Lehtinen, et al. 2011). This preparation maintains embryonic explants in a floating culture system with the apical side of the cortex interfacing with the media through a semipermeable membrane, mirroring the direct exposure of CSF in cortical development.
To investigate roles of the apical environment in supporting cortical development, we cultured E14.5 mouse cortical explants ex vivo across three serum-free media environments: aCSF, Neurobasal Medium, and BrainPhys Neuronal Culture Medium (Bardy, et al. 2015). After 24 and 48 hours, only the explants cultured on BrainPhys Medium mirrored in vivo cortical morphology, recapitulating markers of neural proliferation and cortical lamination. Since BrainPhys Medium mimics the extracellular environment of mature - rather than developing - neurons (Bardy, et al. 2015), we plan to further adjust the composition of the medium according to the physiological levels of neonatal CSF solutes to test additional hypotheses of how other non-protein CSF solutes support neural progenitor proliferation, survival, and differentiation. Together, our findings indicate best practices for cortical explant floating culture and will inform future studies using this powerful technique.

Disclosures: B. Zhou: None. **R.M. Fame:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.25/A23

Topic: A.01. Neurogenesis and Gliogenesis

Support: 2R01MH052716-26 to MMM

Title: Microglia are necessary for retention of a unique and transient mast cell population in the developing brain.

Authors: *K. ENGEL[1], M. M. MCCARTHY[2];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: A transient and proliferative population of mast cells, innate immune cells primarily known for their role in allergic responses through histamine release, briefly reside in the ventricular space between the developing hippocampus and thalamus. Hereafter referred to as peri-hippocampal mast cells (phMCs), they peak in number around postnatal day 7 (PN7) and disappear by PN20. Within this critical window, we found that in Sprague Dawley rats, phMCs undergo piecemeal degranulation thereby releasing factors into the local environment that modulate neurodevelopmental processes. When phMC degranulation is induced on PN2 there is reduced microglial proliferation in the peri-hippocampal space (PMID: 39662467), indicating a functional interaction between phMCs and microglia. Compelling new data indicate that experimental depletion of microglia at PN0 and PN1 using clodronate liposomes (clodronate) leads to a persistent decrease in phMC numbers throughout the first two postnatal weeks compared to empty liposome treated controls (vehicle). Importantly, clodronate does not directly affect MCs through induction of apoptosis nor does it reduce their proliferative capacity. Based on these findings, we **hypothesize that microglia support the residence and maintenance of MCs in the peri-hippocampal region during early postnatal development.** To test this hypothesis, we plan to isolate deep and superficial cervical lymph nodes (dcLNs and scLNs), the rostral migratory stream (RMS), and the sinus epithelium following treatment with vehicle or clodronate, to determine whether in the absence of microglia the phMCs are exiting the brain by migrating through the glymphatic system or through interstitial pathways. We expect to observe an increase in MCs in these tissues when microglia are depleted, suggesting that microglia are secreting a factor that either recruits or encourages residency of the MCs in this peri-hippocampal space. If confirmed, this would be a novel interaction between microglia and canonical peripheral immune cells in early postnatal development with implications for brain health and disease.

Disclosures: K. Engel: None. **M.M. McCarthy:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.26/A24

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH Grant 5R01MH052716-27
T32 AI095190

Title: Peri-hippocampal mast cells influence oligodendrocyte development during the early postnatal period

Authors: *M. R. BRUCE[1], A. ALLEE[2], M. M. MCCARTHY[3];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Univ. of Maryland Baltimore, Ellicott City, MD; [3]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Early life immune activation, including allergy, can result in detrimental effects on neurodevelopment. Of interest, transient populations of immune cells with distinct transcriptional programming exist within the brain and associated border regions during the early postnatal period, including mast cells (PMID: 17764664) and white matter associated macrophages (PMID: 31262353). Activation of these immune cells during sensitive periods of development may contribute to effects seen in models of early life immune challenge. A uniquely proliferative CNS-resident mast cell population exists in the peri-hippocampal area during the first two postnatal weeks of life in rodents. Pharmacological activation of peri-hippocampal mast cells (phMCs) by intraventricular infusion of the specific degranulator c48/80 increases blood-brain barrier permeability, promotes transient monocytic infiltration, and has effects on the local microglial population (PMID: 39662467). Additional roles suggested by analysis of gene expression in phMCs compared to other mast cell populations relate to regulation of insulin growth factor and thyroid hormone signaling, both of which are involved in oligodendrocyte development and myelination. We sought to answer whether mast cell activation affects oligodendrocyte maturation in the hippocampus. To examine this, we injected rat pups intracerebroventricularly (I.C.V.) with either c48/80 or saline from postnatal days 5-7, to model chronic mast cell activation. We then conducted immunofluorescent (IF) staining with oligodendrocyte lineage markers (PDGFRa/BCAS1/CC1) to quantify the effect of phMC degranulation on oligodendrocyte maturation states in the hippocampus, with results suggesting enhanced oligodendrocyte maturation in response to c48/80 exposure compared to controls. We are also examining whether phMC activation has any effect on white matter associated microglia in the corpus callosum, since it is known that this macrophage population is involved in regulation of oligodendrocyte progenitor cell (OPC) number during the early postnatal period. These data will provide insight into a role for phMCs in oligodendrocyte development, which may reveal novel mechanisms of early life inflammation induced changes in the brain.

Disclosures: M.R. Bruce: None. **A. Allee:** None. **M.M. McCarthy:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.27/A25

Topic: A.07. Development of Neural Systems

Support: NIH R01 NS142029
TSC Alliance

Title: Characterization of PI3K-Akt-mTORC1/2 activity in the mouse cortex across embryonic and postnatal development

Authors: D. RODRIGUEZ, *L. NGUYEN;
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Abstract: The PI3K-Akt-mTORC1/2 signaling pathway is a major regulator of many cellular functions, including cell growth, protein synthesis, and autophagy. Mutations along the PI3K-Akt-mTORC1/2 pathway have been identified in individuals with neurodevelopmental disorders characterized by cortical malformations. Expression of these mutations in the mouse cortex recapitulates these developmental abnormalities, further emphasizing an important role for this pathway in cortical development. To better understand the developmental time course of PI3K-Akt-mTORC1/2 signaling in the normal cortex, we examined the protein levels of key pathway components in the mouse cortex across embryonic and postnatal development. We performed Western blot analysis on whole cortical tissue collected at embryonic day (E) 15 and 17, postnatal day (P) 0, 5, 10, 15, 20, and adulthood (n = 7-8 animals/age). The following proteins were analyzed: phospho-S6 (S240/244; readout of mTORC1 activity), phospho-Akt (S473; readout of mTORC2 activity), phospho-Akt (T308), Rheb (positive regulators), PTEN, TSC1, and TSC2 (negative regulators). All protein bands were normalized to total protein levels and statistical differences across time points were analyzed with one-way ANOVA with the significance level set at $p<0.05$. The levels of the mTORC1 and mTORC2 activity readouts, phospho-S6 and phospho-Akt (S473), respectively, were significantly increased at birth compared to embryonic life and peaked between P5 and P15 before decreasing to adult levels. This observation was consistent with similar increases in the levels of the positive upstream regulators, phospho-Akt (T308) and Rheb, and decreases in the negative regulator, TSC1. TSC2 levels remained unchanged. Interestingly, PTEN levels increased during the same time window, suggesting it may be increased to counteract the downstream Akt-mTORC1 activation. Together, our results demonstrate age-dependent changes in key PI3K-Akt-mTORC1/2 components across the span of cortical development in mice. Interestingly, protein and phosphorylation levels peaked during the second week of life, coinciding with a period of rapid dendritic growth and heightened synaptogenesis, warranting further investigation into the role of temporally regulated PI3K-Akt-mTORC1/2 activity in the structural and functional development of the cortex.

Disclosures: D. Rodriguez: None. **L. Nguyen:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.28/A26

Topic: A.07. Development of Neural Systems

Support: DP2 NS122550-01
P01 NS083513
UCSF/UCB Schwab Dyslexia & Cognitive Diversity Center, 2023 Innovation Fund
Weill Award for Junior Investigators in the Neurosciences Impacted COVID-19 Setbacks
Chan Zuckerberg Initiative
UCSF NBRI

Title: Regional specification of the human Temporal Cortex involves distinct and late integration of GABAergic neurons

Authors: *J. KIM[1], J. CHU[1,2], E. VASQUEZ[1], S. PEER[1,2], C. SHERWOOD[3], P. JI[4], D. XU[1], M. F. PAREDES[1];
[2]Neurol., [1]Univ. of California San Francisco, San Francisco, CA; [3]George Washington Univ., Washington, DC; [4]Univ. of California Davis, Davis, CA

Abstract: The human temporal cortex emerges with significant anatomical expansion and regional specification, enabling higher cognitive functions including language processing. The superior temporal gyrus (STG), middle temporal gyrus (MTG), and inferior temporal gyrus (ITG), as subregions of the temporal cortex (TC), are structurally and functionally specialized for auditory, semantic, and visual processing, respectively. Disruptions in TC development have been implicated in neurodevelopmental disorders (NDDs), contributing to symptoms such as sensory hypersensitivities and dyslexia. However, the developmental mechanisms underlying the regional and functional specification of the human TC remain largely unknown. Here, we investigate the developmental trajectories of GABAergic interneurons in the infant TC, a critical neuronal population essential for maintaining neuronal network balance; their disruption has been linked to pathological symptoms in several NDDs. We observed distinct interneuron integrations into STG, MTG, and ITG in developing human brains. Our transcriptomic and histological analyses revealed that these streams originate from different ventricular niches and exhibit distinct molecular identities: migratory streams into STG arise from the dorsal subventricular zone (SVZ) and consist of diverse GABAergic subtypes, whereas MTG and ITG streams, which are from the ventral SVZ, are predominantly populated with caudal ganglionic eminence (CGE)-derived neurons. Furthermore, our profiling of adult human TC revealed significant differences in VIP-interneuron composition and its diversity between STG, MTG, and ITG. These findings suggest that the differential migratory trajectories of GABAergic interneurons contribute to the regional specification of the human TC, with implications for understanding their functional specifications and related vulnerabilities in NDDs.

Disclosures: J. Kim: None. **J. Chu:** None. **E. Vasquez:** None. **S. Peer:** None. **C. Sherwood:** None. **P. Ji:** None. **D. Xu:** None. **M.F. Paredes:** None.

Poster

PSTR053: Neuronal Development Across Systems: Molecular Mechanisms of Growth, Migration, and Maturation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR053.29/A27

Topic: A.07. Development of Neural Systems

Title: Identifying the Spatiotemporal Origin of CGE-derived Interneuron Subtypes

Authors: *V. GARG[1], A. TANZILLO[1], S. BEYENE[2], A. TUCKER[5], A. JOHANTGES[6], Y. ZHANG[3], T. J. PETROS[4];
[1]Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD; [3]NICHD, [2]NICHD/Eunice Kennedy Shriver Nat'l Inst. of Child H, Bethesda, MD; [4]Developmental Neurosci. Br., NICHD/Eunice Kennedy Shriver Nat'l Inst. of Child H, Rockville, MD; [5]Natl. Inst. for Child Hlth. and Human Develop., Bethesda, MD; [6]Dept. of Neurosci., The Ohio State Univ., Columbus, OH

Abstract: The mammalian cortex consists of a balance of inhibitory GABAergic cortical interneurons (cINs) and excitatory neurons. Dysfunction of this balance contributes to a series of neurodevelopmental disorders such as epilepsy, ASD, schizophrenia. cINs originate from transient structures in embryogenesis known as the ganglionic eminences (GEs)-specifically, the medial (MGE), caudal (CGE), and lateral ganglionic eminences (LGE). Each GE produces different cIN subtypes of varying transcriptomic, morphological, and electrophysiological characteristics. Previous experiments have established a spatiotemporal framework for cells within the MGE, whereby a combination of regional origin and temporal birthdate shape cIN fate. Comparatively less is known about the contribution of the CGE to the development of its specific cIN subtypes defined by expression of vasoactive intestinal peptide (VIP), cholecystokinin (CCK), calretinin (CR), and reelin (Reln). Here, we harvest cells from the anterior, posterior, dorsal, and ventral CGE at E13.5 and E15.5 from *Dlx-Cre;Ai9* mice and transplant them into WT neonatal mice. One month post-transplantation, after the transplanted cells had migrated throughout the host cortex and reached maturity, we harvested the grafted brains and immunostained them for the four CGE-derived subtypes. Our results indicate that there are significant spatial biases in the CGE, with specific interneuron subtypes preferentially arising from distinct CGE subdomains. This effect is most pronounced for VIP+ and Reln+ interneurons, which are strongly enriched in dorsal and posterior transplants, respectively. Interestingly, scRNAseq analysis of adult mouse brain cortex reveals that these two subtypes occupy largely non-overlapping, complementary clusters within the CGE lineage-a relationship similar to that of the major subtypes originating from the MGE. These regional biases are relatively stable over time, implying a minimal relationship between temporal birthdate and

interneuron subtype. In the future, combining these insights with spatial transcriptome profiles will generate critical insights into gene regulation of CGE-derived interneurons.

Disclosures: V. Garg: None. **A. Tanzillo:** None. **S. Beyene:** None. **A. Tucker:** None. **A. Johantges:** None. **Y. Zhang:** None. **T.J. Petros:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.01/A28

Topic: A.04. Transplantation and Regeneration

Support: NIH Pioneer Award DP1 NSI06665
Travis Roy Foundation
NIH T32AG000222
NIH F30HD113352
Fondation Jean-Jacques et Felicia Lopez-Loreta pour excellence académique
HCBI Simmons Award
NIH MSTP T32GM007753

Title: Dynamic regulation of subcellular molecular machinery of developing and regenerating corticospinal growth cones

Authors: K. NAIRN[1], ***M. A. VICENT**[2,1], A. ENGMANN[1], J. FROBERG[1], D. TILLMAN[1], D. NGUYEN[3,1], O. DURAK[1], J. D. MACKLIS[1];
[1]Dept of Stem Cell and Regenerative Biology, and Ctr. for Brain Sci., [2]Harvard Univ., Cambridge, MA; [3]Loyola Univ. Chicago, Forest Park, IL

Abstract: Corticospinal neurons (CSN) enable voluntary movement and dexterous motor control. Their cell bodies reside in layer V of neocortex. During development, CSN extend axonal projections 1K-100K cell body diameters away from their somata, to the brainstem and spinal cord, to establish precise circuitry. Injury of CSN circuitry, such as in spinal cord injury (SCI), causes critical and irreversible loss of motor function due to lack of CSN regeneration. Due to extreme polarity, neurons contain multiple distinct subcellular transcriptomes, with local protein synthesis of axonally-enriched transcripts required for directional responses to at least some guidance cues, and for formation of presynaptic terminals. However, the composition of subtype-specific growth cone (GC)-localized molecular machinery and the dynamic regulation of these subcellular processes across distinct stages of circuit formation are essentially unknown. Discovery and investigation of animal models of increased CSN regeneration, e.g. *Pten* deletion, has led to poor translational progress for humans with SCI, partly due to lack of mechanistic understanding of axon and GC-localized processes enabling regeneration. We apply a combination of neuronal subtype-specific labeling, biochemical fractionation, and fluorescent

small particle sorting for transcriptomic, translatomic, and proteomic analyses of CSN-specific GCs and somata. This enables investigation of dynamic regulation of subcellular CSN molecular machinery in response to changing, complex, *in vivo* environments, both during development and in response to injury. Multiomic analyses across developmental stages identify suites of genes regulated transcriptionally and post-transcriptionally as neurons transition from early, axon-elongating growth phenotypes, to later, innervating/branching phenotypes. We employ similar subcellular strategies with genetically modified, regenerating CSN axons and somata after thoracic SCI to discover local regenerative growth programs. Comparison of developing and regenerating CSN GCs will deepen understanding of regenerative growth. In particular, to what extent regenerative growth reuses subcellular mechanisms of developmental growth, and how the adult, injured cord constrains regenerating neurons to employ distinct mechanisms for regeneration.

Disclosures: K. Nairn: None. **M.A. Vicent:** None. **A. Engmann:** None. **J. Froberg:** None. **D. Tillman:** None. **D. Nguyen:** None. **O. Durak:** None. **J.D. Macklis:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.02/A29

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant NS089633

Title: Reg3a to khsrp signaling restricts cns axon regeneration

Authors: *S. MATOO[1], J. LEE[2], C. N. BUCHANAN[1], L. S. VAUGHN[1], J. L. TWISS[1]; [1]Univ. of South Carolina, Columbia, SC; [2]Northwestern Univ., Chicago, IL

Abstract: Neurons of adult CNS have a limited ability to regenerate, restricting functional recovery after injury. In contrast, axons in the PNS have an intrinsic capacity for spontaneous regeneration, supported in part by localized mRNA translation that enables local synthesis of growth-promoting proteins. Increasing evidence shows that CNS axons contain mRNAs and synthesize proteins. We previously reported that the RNA-binding protein KHSRP is locally translated in injured PNS axons, where it limits regeneration by promoting mRNA decay. Although KHSRP translation is initially triggered by injury-induced axoplasmic Ca^{2+} influx, KHSRP protein remains elevated long after Ca^{2+} levels normalize, suggesting a mechanism to sustain axonal KHSRP synthesis. We have shown that REG3A, a secreted lectin-like protein, increases axoplasmic Ca^{2+} and recombinant REG3A (recREG3A) increases axonal KHSRP and inhibits axon growth in DRG cultures. Depletion of endogenous REG3A decreases axoplasmic Ca^{2+} and increases axon growth and PNS nerve regeneration. Using optic nerve (ON) crush as a model of CNS injury, we find upregulation of *Reg3a* mRNA in injured ON axons, and increased REG3A and KHSRP proteins in injured ON. To test whether REG3A suppresses CNS

regeneration, we injected AAV2-shReg3a-BFP or AAV2-shControl-BFP intravitreally to target retinal ganglion cells (RGCs). Depletion of RGC Reg3a enhanced axon regeneration after ON crush injury and increased RGC survival compared to controls. CNS growth inhibitory substrates increase axoplasmic Ca^{2+} and elevate axonal KHSRP levels. REG3A-depleted DRG neurons extend axons and do not show elevation of axonal KHSRP when cultured on CNS growth-inhibitory substrates. Together, these findings show that REG3A-KHSRP signaling intrinsically limits CNS axon regeneration and likely enhances neuronal sensitivity to growth-inhibitory substrates.

Disclosures: **S. Matoo:** A. Employment/Salary (full or part-time): University of South Carolina. **J. Lee:** A. Employment/Salary (full or part-time): Northwestern University. **C.N. Buchanan:** A. Employment/Salary (full or part-time): University of South Carolina. **L.S. Vaughn:** A. Employment/Salary (full or part-time): University of South Carolina. **J.L. Twiss:** A. Employment/Salary (full or part-time): University of South Carolina.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.03/A30

Topic: A.04. Transplantation and Regeneration

Support: NRF 2022R1A2C2092821
MSIT RS-2024-00333219

Title: Importance of electrode placement in electrical stimulation to promote wound regeneration: a convergent study in preclinical and simulation models

Authors: ***S. KIM**[1], D. KANG[2], I. KIM[3], E. PARK[1];
[1]SOONCHUNHYANG UNIVERSITY, Asan, Korea, Republic of; [2]Dept. of Biomed. Engin., [3]?????, Hanyang university, Seoul, Korea, Republic of

Abstract: Wound regeneration is essential for survival. Unhealed wounds can lead to serious complications such as sepsis and tissue necrosis, thereby reducing quality of life. Chronic wounds also decrease occupational productivity and impose significant economic burdens due to prolonged recovery and increased medical costs. The importance of effective wound regeneration is further underscored by the rising prevalence of diabetes and an aging population, both of which are associated with a higher incidence of chronic wounds. Endogenous electric fields (EF) have been shown to induce electrotaxis, a process in which cells migrate directionally toward wound sites, thereby accelerating regeneration. Applying an exogenous EF to stimulate transcutaneous nerves, along with enhancing endogenous EF, can effectively promote cell migration and wound regeneration. However, electric field formation through transcutaneous electrical nerve stimulation depends on electrode placement, a factor that remains insufficiently studied. This study evaluated the effect of electrode positioning on wound regeneration via

preclinical experiments and analyzed corresponding EF distributions using computational simulations. The relationship between simulated EF gradients and biological outcomes was then assessed. In preclinical experiment, a full-thickness skin defect rat model was created using a biopsy punch, assigning one control and three treatment groups with different electrode placements. High voltage pulsed current (HVPC), considered highly effective for wound regeneration, was applied for stimulation. Over 17 days, wound regeneration was monitored through wound area analysis and histological evaluation using Hematoxylin and Eosin staining. Results showed wound areas of 26% in the control group versus 16%, 17%, and 9% in treatment groups, indicating statistically significant improvement. Histological analysis further confirmed significant differences in gap of epithelium and granulation tissue, demonstrating the influence of electrode placement. Simulations conducted using COMSOL Multiphysics, with skin-like material properties, revealed variations in EF gradients, a key factor in directing cell migration. Notably, current overcrowding was observed at specific placements, suggesting potential heat generation that may hinder wound regeneration. The correlation between simulation and experimental findings provides quantitative evidence of the impact of EF distribution. These results suggest guidelines for optimal electrode placement and offer insights into improving electrical nerve stimulation strategies for wound regeneration.

Disclosures: S. Kim: None. **D. Kang:** None. **I. Kim:** None. **E. Park:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.04/A31

Topic: A.04. Transplantation and Regeneration

Support: CIHR project grant 165839

Title: Co-transplantation of neural and endothelial stem cells promotes neurovascular regeneration after ischemic stroke in a rat model.

Authors: *D. SARAF[1,4], A. CARTER[2], J. WANG[4,3];
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Abstract: Current treatments for ischemic stroke (IS) are limited and do not address brain tissue regeneration, leaving a critical therapeutic gap. Neural stem cell (NSC) therapy shows promise, but poor cell survival in the post-stroke microenvironment restricts its efficacy. Our lab previously demonstrated the neuro-regenerative potential of metformin-preconditioned human induced pluripotent stem cell-derived NSCs (iPSC-NSCs), though recovery remained limited. Given that stroke pathology includes both neuronal loss and vasculature damage, we

hypothesized that co-transplanting metformin-preconditioned human NSCs (hNSCs) and human endothelial colony-forming cells (hECFCs) at the post-stroke subacute stage would better support post-stroke neurovascular unit (NVU) regeneration and functional recovery. Using in vitro assays, we identified 100 μM metformin as the optimal preconditioning dose, enhancing proliferation (EdU staining) and differentiation of both hNSCs (βIII-tubulin immunostaining) and hECFCs (tube formation), indivdually. In co-culture, metformin preconditioning produced additive pro-differentiation effects on both hNSC and hECFCs. In vivo, we co-transplanted metformin-preconditioned cells into a rat endothelin-1 (ET-1) stroke model one-week post-injury. Engraftment success was highest in the co-transplant group (metformin-preconditioned both hNSC+hECFC) (n=8/8 rats), compared to single-cell (metformin-preconditioned hNSC) (n=6/8) and non-preconditioned co-transplant group (hNSC+hECFCs) (n=1/5) groups. Surviving grafts showed integrated vasculature and neurogenesis: hNSCs differentiated into DCX+ neurons, and hECFCs integrated into host vessels. Host vasculature and glia integrated with graft cells, suggesting reconstitution of a functional NVU. These preliminary findings support the therapeutic potential of metformin-preconditioned hNSC+hECFC co-transplantation by boosting neurovascular repair for post-stroke functional recovery.

Disclosures: D. Saraf: None. **A. carter:** None. **J. Wang:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.05/A32

Topic: A.04. Transplantation and Regeneration

Support: 1R01EY033000
1R01EY034325
1K08EY027458
1K08EY032991
FFSGIA16002
P30 EY007003

Title: Multimodal Long-Term Tracking of hiPSC Stem Cells Differentiated to RPE for Regenerative Therapy in Retinal Neurodegenerations

Authors: *P. NGUYEN[1], W. QIAN[2], Y. PAULUS[3], A. FAHIM[4];
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Abstract: Retinal degenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are major causes of irreversible vision loss worldwide, many due to progressive dysfunction and death of retinal pigment epithelium (RPE) cells. Stem cell-based

therapies, particularly using human-induced pluripotent stem cell differentiated to RPE (hiPSC-RPE) cells, offer a promising strategy for cellular replacement and vision restoration. To advance clinical translation, it is critical to develop tools that enable long-term, non-invasive monitoring of transplanted cells in vivo. In this study, we established a rabbit model of RPE damage using a 532 nm Nd:YAG laser (800 mW, 500 μm, 100 ms, 12 spots/eye) and transplanted 10^5 hiPSC-RPE cells labeled with a novel chain-like gold nanoparticle cluster probe conjugated with a nuclear localization sequence peptide and indocyanine green. A custom multimodal imaging system integrating photoacoustic microscopy (PAM), optical coherence tomography (OCT), and fluorescence microscopy was used to track cell location, migration, and tissue integration for up to six months. PAM enabled high-contrast imaging of transplanted cells distinguished from native vasculature, especially at 650 nm. OCT demonstrated progressive structural repair of RPE lesions. Fluorescence imaging corroborated PAM data on cell localization but for a more limited time up to 28 days. Histological and immunofluorescence analyses confirmed engraftment and partial restoration of the RPE layer. This study highlights a powerful imaging platform for lifetime tracking of stem cell therapies in the retina and supports the therapeutic potential of hiPSC-RPE transplantation for treating retinal neurodegeneration. These findings are critical for advancing regenerative strategies aimed at preserving vision and enhancing quality of life in aging populations.

Disclosures: P. Nguyen: None. **W. Qian:** None. **Y. Paulus:** None. **A. Fahim:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.06/A33

Topic: A.04. Transplantation and Regeneration

Support: University of Oslo

Title: Fostering a permissive environment for successful brain organoid transplantation in stroke.

Authors: *T. F. MERGIYA, M. NARUM, J. E. RINHOLM, S. M KANSE;
Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway

Abstract: Stroke is one of the leading causes of death, disability, and dementia in adults. Limited neuronal regeneration poses a significant barrier to recovery. Brain organoids, three-dimensional self-organizing and self-renewing stem cell cultures—offer a promising avenue for regenerative therapies. While organoid transplantation into healthy, young rats and mice has shown success, its efficacy in the stroked brain of adult mice remains uncertain, particularly regarding long-term survival and maturation. Inflammatory/immune responses, impenetrable scar tissue, and limited vascularization at the injury site may impede organoid integration into the host brain. We hypothesize that suppressing the innate immune response, degrading scar tissue, and enhancing angiogenesis within the organoid will improve its survival, maturation, and

functional integration, thereby promoting brain repair. To this end, we induced stroke in adult non-obese diabetic severe combined immunodeficient mice using the photothrombosis method. The mice exhibited motor deficits in the grid-walk test. Triphenyl tetrazolium chloride (which marks viable tissue) staining, Nissl staining, and Evans blue leakage confirmed extensive brain damage. Seven days post-stroke, we performed a craniotomy over the damaged area and transplanted a 50-day-old human brain organoid. Then, we stained the brains for various human cell types, host immune responses, and scar tissue formation. The transplanted organoids survived for up to three weeks with residual human proliferating and progenitor cells, significant microglial infiltration, and scar tissue formation around the graft. Notably, spontaneous sensorimotor recovery occurred in the mice, independent of the transplantation. We observed significant atrophy of the transplanted organoid four weeks after transplantation. These findings highlight persistent challenges of achieving successful brain organoid transplantation for stroke recovery. Strategies to mitigate immune responses, reduce scar tissue, and enhance vascular integration are likely to be critical for improving organoid survival and therapeutic efficacy.

Disclosures: T.F. Mergiya: None. **M. Narum:** None. **J.E. Rinholm:** None. **S. M Kanse:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.07/A34

Topic: A.04. Transplantation and Regeneration

Support: Japan Agency for Medical Research and Development (AMED) JP22bm0704046
Japan Agency for Medical Research and Development (AMED) JP25ym0126168
MEXT KAKENHI 24K19570
JA Niigata Kouseiren Grant (Niigata University School of Medicine)
General Insurance Association of Japan
JST SPRING Niigata University Next Generation Project

Title: Novel Biosafe Engineered Acellular Nerve Grafts: Utilizing Human iPSC-Derived Neurite Bundles from Nerve Organoids

Authors: *K. OKUYAMA[1], T. NISHIJIMA[2,4], J. NAKAYAMA[1], T. Q. PHAN[1], E. NIIYAMA[1,5], J. KAWADA[1,5], S. SHIBATA[1,3];
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Abstract: Severe peripheral nerve injuries remain a major clinical challenge, often necessitating transplantation-based therapies. Autologous nerve grafts are the gold standard but have drawbacks, including donor-site morbidity and size constraints. Although artificial nerves are available, their therapeutic effect remains limited to short gaps in thin sensory nerves. To overcome these challenges, we developed a novel artificial nerve graft composed of human iPSC-derived neurite bundles. Using a custom-designed microfluidic culture device, we generated nerve organoids with bundled neurites exceeding 20 mm length (Kawada J et al., 2017 Stem Cell Rep). These neurite bundles can be separated from their somata, improving biosafety compared with cell grafts. The neurite bundles were aligned within a tube filled with collagen gel and transplanted into large sciatic nerve defects (13 mm) in rats. Twelve weeks after transplantation in wild-type rats, enhanced regeneration of motor and sensory functions was observed in animals receiving neurite-containing grafts compared to controls receiving only collagen gel, without excessive inflammation (Nishijima, Okuyama et al., 2024 Inflamm Regen). Across various analyses, favorable outcomes were confirmed by significant improvements in motor function-related assessments (sciatic function score; recovery of innervated muscle), sensory function-related assessments (Hargreaves test; von Frey test), and histological evaluation of the regenerated nerve (axon number and thickness; myelin quality). In contrast, transplantation into immunodeficient rats resulted in minimal recovery, indicating the host immune response contributes to our neurite graft-mediated regeneration. Early post-transplantation (1-2 weeks) assessments revealed an enhanced regenerative environment with increased infiltration of M2-polarized macrophages, accelerated angiogenesis, and axonal extension. These hallmarks arose with complete degradation of the grafts. Together, these findings suggest that iPSC-derived neurite bundles function as a bioactive scaffold, establishing a pro-regenerative microenvironment. Additionally, transplantation of homogenized neurite fragments in collagen gel yielded similar histological outcomes to intact neurite bundles. This implies that the critical regenerative cue is likely the bioactive molecules within the neurites, whereas intact fiber structure may still be beneficial when bridging longer nerve gaps. Our ongoing studies for clinical application aim to elucidate mechanisms promoting regeneration and identify specific molecular factors using rat and large-animal models.

Disclosures: K. Okuyama: None. **T. Nishijima:** None. **J. Nakayama:** None. **T.Q. Phan:** None. **E. Niiyama:** A. Employment/Salary (full or part-time): Jiksak Bioengineering, Inc. **J. Kawada:** A. Employment/Salary (full or part-time): Jiksak Bioengineering, Inc.. **S. Shibata:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.08/A35

Topic: A.04. Transplantation and Regeneration

Title: Biomaterial-directed patterning of human cortical organoids transplanted into rodent cortex

Authors: *K. E. HERREMA[1], E. K. KHARITONOVA[2], E. M. DUBOIS[1], M. G. SIMKULET[1], A. DEVOR[1,3], M. THUNEMANN[1], E. ZELDICH[2], T. M. O'SHEA[1];
[1]Biomed. Engin., [2]Anat. & Neurobio., Boston Univ., Boston, MA; [3]Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA

Abstract: Human induced pluripotent stem cell (hiPSC)-derived cortical organoids (hCOs) are three-dimensional neural cell aggregates that resemble the developing human cortex and have emerged as an avenue to study pathological processes in a human-centric experimental context. Previous work by us and others demonstrated that transplanting hCOs into rodent brain leads to xenograft vascularization and facilitates maturation, synaptic connectivity, and integration of human neurons into host neuronal networks. However, disruption of hCO cytoarchitectures and high graft-to-graft variability following transplantation limit the interpretability of these models. To permit widespread use of this technology, there is a critical need to direct the neuronal patterning of xenografted hCOs in vivo. The use of spatiotemporal morphogen gradients has previously been demonstrated to direct hCO cytoarchitectures in vitro. In this work, we aim to apply morphogen gradients in vivo to direct xenograft neuronal patterning and maturation, enabling robust biological models for the study of human neurodevelopment and disease. Toward this end, we developed HEC_{MTP}, a cellulose-based film, for in vivo morphogen gradient delivery. We co-transplanted hCOs into the adult mouse retrosplenial cortex alongside HEC_{MTP} films loaded with retinoic acid (RA), a potent developmental morphogen that plays a key role in cortical development and laminar patterning. To monitor neuronal function, human neurons were labelled with GCaMP8s prior to transplantation. One month after transplantation, two-photon calcium imaging indicated increased neuronal activity adjacent to the film compared to those at further distances from the film as well as an overall increase in neuronal activity compared to xenografts without RA gradient exposure. These results show that providing an exogenous RA gradient can spatially regulate neuronal fate and advance neuronal maturation of hCOs in vivo. Post-mortem immunostaining revealed that neurons surrounding the film surface present an upper-layer neuronal identity (SATB2+) compared to neurons further from the film which expressed more lower-layer markers (CTIP2+). RA gradient-exposed xenografts also maintained smaller, more consistent sizes whereas control xenografts exhibited extensive growth resulting in the depression of host brain structures such as the hippocampus. These results indicate that a prolonged RA gradient provided by a novel biomaterial film platform can successfully be applied to regulate neuronal patterning in xenografted hCOs and improve graft-to-graft consistency in size and integration.

Disclosures: K.E. Herrema: None. **E.K. Kharitonova:** None. **E.M. DuBois:** None. **M.G. Simkulet:** None. **A. Devor:** None. **M. Thunemann:** None. **E. Zeldich:** None. **T.M. O'Shea:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.09/A36

Topic: A.04. Transplantation and Regeneration

Support: Advancing a Healthier Wisconsin (AHW)

Title: Hallmarks of hypertranscription are present in zebrafish retinal ganglion cells during axon regeneration

Authors: *H. G. TEBBS, M. B. VELDMAN;
Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Hypertranscription is a cellular state characterized by a global, non-selective surge in gene expression. It has been documented in cancer, T-cell activation, and development states of rapid growth and increased plasticity where biosynthetic demand is elevated. The biosynthetic demand imposed by axon regeneration in neurons is likely similar. However, the relevance of hypertranscription after nerve injury has yet to be evaluated in the context of central nervous system (CNS) regeneration. Mammalian models of CNS injury fail to mount a regenerative response hindering our ability to identify factors needed for successful regeneration. In contrast, zebrafish possess the unique ability to recover from CNS injuries and restore function, making them an ideal model to study the cellular changes associated with axonal regeneration. In this study, we use the zebrafish optic nerve crush model to determine whether retinal ganglion cells (RGCs) utilize hypertranscription to successfully regenerate their axons. The hallmarks of hypertranscription include increased total RNA output, intensified ribosome biogenesis, widespread chromatin accessibility, upregulated universal amplifier expression, and greater evidence of transcription linked DNA stress. To explore the presence and functional role of this process we isolated RGCs by FACS and measured total RNA normalized to cell number in naïve and 10 day post optic nerve injury regenerating cells. We observed a significant increase in total RNA content and nuclear volume during axon regeneration, consistent with a transient hypertranscriptional state. qRT-PCR established an increase in ribosomal RNA expression and select ribosomal protein transcripts during axon regeneration. Furthermore, transcripts coding for MYC family universal amplifiers were also upregulated following the optic nerve crush, suggesting that these factors help mediate the transcriptional program necessary for regeneration. These findings demonstrate that regenerating zebrafish RGCs display three key hallmarks associated with hypertranscription: enhanced RNA production, increased ribosome biogenesis, and elevated MYC expression. Future work will test for the remaining hallmarks of increased chromatin accessibility and transcription related DNA damage. Our data support the hypothesis that hypertranscription is occurring during axon regeneration in the zebrafish CNS. Activation of hypertranscription may stimulate regeneration in mammalian neurons.

Disclosures: H.G. Tebbs: None. **M.B. Veldman:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.10/A37

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01EY034097
NIH Grant R01EY034097-03S1

Title: The timeline of retinal ganglion cell axonal degeneration and unesterified cholesterol availability after optic nerve injury in the adult zebrafish

Authors: *H. JURKIEWICZ[1], M. B. VELDMAN[2];
[2]Cell Biology, Neurobiology, and Anat., [1]Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Cholesterol accumulation and improper trafficking have been associated with multiple chronic neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease. Cholesterol accumulation in the mammalian central nervous system (CNS) has been implicated in both neurotoxicity and inhibition of axon regeneration following injury or degeneration. The synthesis and trafficking of cholesterol in proregenerative vertebrates such as adult zebrafish (Danio rerio), are not well described. Thus, we are investigating cholesterol trafficking and accumulation in the adult zebrafish visual system following optic nerve (ON) injury to understand how cholesterol is managed during successful CNS regeneration. We hypothesize that unesterified cholesterol obtained from the degenerating ON following optic nerve crush (ONC) is reutilized by the retinal ganglion cells (RGC) to facilitate successful axonal regeneration. Therefore, we examined the availability of unesterified cholesterol in the ON following ONC by performing a cholesterol stain, Filipin, across the time course of early ON regeneration (0 to 72 hours post-injury (hpi)). Our results demonstrate that cholesterol accumulation reaches its highest peak by 6 hpi, suggesting the presence of unesterified cholesterol shortly after injury. Interestingly, interestingly, our results show no change in filipin intensity by 24 hpi, when compared to baseline. These findings suggest that unesterified cholesterol is elevated shortly after injury throughout the degenerating ON and is regulated by clearing by 24 hpi. Next, to determine if RGC axonal degeneration contributes to theobserved changes in unesterified cholesterol, we examined the time course of RGC axonal degeneration in isl2b:GFP transgenic zebrafish. Using whole brain tissue clearing, we imaged RGC axonal projections in the optic tectum (OT) after ONC. Axonal fragmentation was observed as early as 18 hpi. Total axonal area in the OT is reduced by 25% by 24 hpi, and more than 70% of the total area is lost by 72 hpi. These results suggest that RGC axons degenerate after injury and follow the stages of Wallerian degeneration. Altogether, we have demonstrated novel timelines for the progression of RGC axonal degeneration, as well as established the presence of unesterified cholesterol throughout the ON. Surprisingly, axon degeneration does not temporally correlate with the availability of unesterified cholesterol in the ON. This suggests alternative cholesterol sources, such as acutely injured oligodendrocytes or vascular leakage. We are currently examining the mechanism(s) of cholesterol re-uptake after ONC and the cell types responsible.

Disclosures: H. Jurkiewicz: None. **M.B. Veldman:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.11/A38

Topic: A.04. Transplantation and Regeneration

Support: Upstate Foundation, A New Beginning Fund

Title: Comparison of Schwann cell growth properties and repair phenotypes from different species

Authors: K. KRICK[1], *D. J. OSTERHOUT[2];
[1]Norton Col. of Med., [2]Dept. of Cell and Developmental Biol., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Schwann cell transplantation has shown promise in enhancing nerve repair in both the peripheral and central nervous systems. After a nerve injury, Schwann cells will de-differentiate and adopt a repair phenotype, secreting multiple factors which stimulate axonal growth and remyelination. Given this response, Schwann cells have been included in nerve grafts or guidance channels that are used to promote repair after spinal cord or peripheral nerve injury models. The Schwann cells utilized are most often autologous, isolated from a small nerve segment, expanded in culture, and implanted into the lesion. Many of these studies have been conducted in rodent models of injury, with rodent Schwann cells. However, there is evidence that rodent Schwann cells have different growth properties than human Schwann cells. Thus, the repair obtained in rodent models may not be readily obtained in human nerve lesions. For this reason, injury models are being developed in other species, including porcine and canine peripheral nerves. These are thought to be more physiologically representative models of human nerve injury, with Schwann cells that behave more similarly to human Schwann cells. However, the cellular properties of porcine and canine Schwann cells have not been well described, especially after injury. In this study, we describe the growth properties of Schwann cells from several species, particularly in regard to their growth on different substrates that might be utilized in a guidance channel. We examined cell proliferation, migration, and the ability of Schwann cells over time to maintain a repair phenotype, which may significantly influence their ability to stimulate axon regrowth over time.

Disclosures: K. Krick: None. **D.J. Osterhout:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.12/A39

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant EY031403
Maine INBRE

Title: Development and synapse formation in induced retinal ganglion cells

Authors: C. ZIEHL, R. LUTZ, L. STERBA, ***M. B. WOODWORTH**;
Bates Col., Lewiston, ME

Abstract: Retinal ganglion cells (RGCs) are neurons that relay visual information from the retina to the brain. The adult mammalian retina cannot regenerate, so RGC injury or death results in irreversible visual impairment. In previous work, we found that in vivo electroporation of a set of candidate transcription factors induces the generation of RGC-like neurons outside the window of endogenous RGC development in early postnatal mice. Although these RGC-like neurons send long-distance axons to visual targets, they are unable to successfully form synaptic connections with neurons in lateral geniculate nucleus of thalamus. To allow induced RGCs to establish synaptic connections, we hypothesized that we must disrupt synapses made by endogenous RGCs, which navigate to visual targets and establish synapses weeks before induced RGC axons do. Further, we investigated transcription factors regulating the development of both endogenous and induced RGCs. Through this work, we aim to produce circuit-integrated RGCs induced outside the normal window of RGC development, an important step toward regenerating RGCs after disease or injury.

Disclosures: C. Ziehl: None. **R. Lutz:** None. **L. Sterba:** None. **M.B. Woodworth:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.13/Web Only

Topic: A.04. Transplantation and Regeneration

Title: Neuronal Regeneration Alternatives in the Peripheral Nervous System: preliminary findings of a literature review.

Authors: R. GARCIA GONZALEZ[1], M. GARCÍA GONZÁLEZ[2], R. CORTÉS MONTES DE OCA[4], A. CHAVEZ MANRÍQUEZ[5], J. GONZÁLEZ DELGADO[4], I. GARCÍA PAYÁN[6], M. VEGA GARCÍA[4], M. TOVANY RAMIREZ[4], K. REYES VILLANUEVA[7], S. RENDÓN ALAMEDA[7], E. MELCHOR FOSADO[7], J. ZECENARRO MAMANI[8], A. FLORES GARCÍA[3], ***I. PEREZ-NERI**[1];
[1]Natl. Inst. of Rehabil. Luis Guillermo Ibarra Ibarra, Mexico City, Mexico; [2]Benemérita Univ. Autónoma de Puebla, San Pedro Cholula, Mexico; [3]Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [4]Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [5]Univ. Popular del Estado Puebla, Puebla, Mexico; [6]Med. Synthesis Intl. Sch., CDMX, Mexico; [7]Univ. de la Salud del Estado de Puebla, Puebla, Mexico; [8]Univ. Católica Boliviana San Pablo, Santa Cruz, Bolivia, Plurinational State of

Abstract: Introduction. In the regeneration of the peripheral nervous system (PNS), support cells primarily Schwann cells—play a key role by promoting axonal growth through the indirect mediation of neurotrophic factors[1]. Currently, innovative surgical techniques have been developed, such as the use of transplants and scaffolds incorporating cellular components or substitutes[2]. **Methods.** A literature review was conducted in PubMed using algorithms based on Medical Subject Headings (MeSH): "Nerve regeneration" and "Peripheral Nervous System Diseases." Preclinical, clinical, and review studies on neuronal regeneration techniques were included. Excluded were theoretical reviews, studies focused solely on pathophysiological aspects, diagnostic techniques, or treatments unrelated to PNS regeneration. **Results.** A total of 57 articles published between 2001 and 2025 were retrieved, identifying the following therapeutic alternatives: transplants, grafts, conduits, implants, pharmacological agents, devices, gene therapy, techniques involving support cells, and strategies using neurotrophic factors. **Discussion.** Autologous transplants remain the cornerstone of treatment, though their efficacy improves when combined with scaffolds enriched with stem cells or neurotrophic factors. Mesenchymal and bone marrow-derived stem cells stand out due to their versatility and clinical feasibility, while gene therapy, though promising, still requires vector optimization and long-term safety validation. Neuroprotective drugs and photobiomodulation serve as complementary approaches, enhancing neuronal viability in multimodal strategies. **Conclusion.** According to the literature, achieving optimal therapeutic outcomes requires the simultaneous application of multiple techniques. Therefore, combining direct approaches—such as transplants—with indirect methods—such as stimulation of support cells—may offer improved efficacy in PNS regeneration. **References.**1. Gordon T. Nerve regeneration in the peripheral and central nervous systems. JPhysiol. 2016 Jul 1;594(13):3517-20. doi: 10.1113/JP270898. PMID: 27365158; PMCID: PMC4929322. 2. Xiaosong Gu, Fei Ding, Yumin Yang, Jie Liu. Tissue Engineering in Peripheral Nerve Regeneration En: So KF & Xu XM. Neural Regeneration. United States of America: Elsevier; 2015. 73-99.

Disclosures: R. Garcia Gonzalez: None. **M. García González:** None. **R. Cortés Montes de Oca:** None. **A. Chavez Manríquez:** None. **J. González Delgado:** None. **I. García Payán:** None. **M. Vega García:** None. **M. Tovany Ramirez:** None. **K. Reyes Villanueva:** None. **S. Rendón Alameda:** None. **E. Melchor Fosado:** None. **J. Zecenarro Mamani:** None. **A. Flores García:** None. **I. Perez-Neri:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.14/A40

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant EY031403
Maine INBRE

Title: Effects of Gap43 and Reelin on Retinal Ganglion Cell Axon Pathfinding

Authors: *K. E. BURNHAM[1], M. B. WOODWORTH[2], K. RENTZEPIS[1];
[2]Neurosci., [1]Bates Col., Lewiston, ME

Abstract: Retinal ganglion cells (RGCs) are projection neurons that connect the retina with the visual processing areas in the brain. Given that the adult mammalian retina is unable to regenerate RGCs, loss of these neurons via injury or disease is irreversible and devastating. Previous work in our laboratory has established a set of developmentally expressed transcription factors sufficient to induce the generation of RGCs from late progenitors in the mouse retina. Axons of these induced RGCs extend into the optic nerve, then progressively reach central visual targets including the thalamus and superior colliculus. In this work, we aim to understand how these axons navigate in the early postnatal nervous system, when cues that guided their endogenous counterparts are no longer available. Gap43 is upregulated in induced RGCs compared with control retinal neurons; Gap43 encodes a protein expressed at high levels in neuronal growth cones during development and implicated in active axon growth and synaptogenesis. Reelin is a glycoprotein that helps neurons migrate to their proper laminar position during nervous system development and may function in axon guidance. Its role in neuronal organization aids in proper building and functioning of the brain. By investigating Gap43 and Reelin in the context of RGC regeneration, we hope to better understand how axon guidance molecules affect the generation and development of RGCs from existing cells intrinsic to the retina, which could enable restoration of vision in human patients.

Disclosures: K.E. Burnham: None. **M.B. Woodworth:** None. **K. Rentzepis:** None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.15/A41

Topic: A.04. Transplantation and Regeneration

Support: P20GM103423
P20GM104318
P20GM144265

Title: Neural crest-like cell transdifferentiation underlies a new mode of neuronal regeneration in the zebrafish retina

Authors: *R. MADELAINE;
MDI Biol. Lab., BAR HARBOR, ME

Abstract: In humans, retinal neurons death, optic nerve injuries and associated neurodegenerative diseases, such as glaucoma or age macular degeneration, often lead to permanent loss of vision. While the regenerative capacity is low in the human nervous system, including retina, the endogenous neuronal regenerative process after injury occurs in some non-mammalian vertebrate species, like the zebrafish. Unlike mammals, zebrafish do not form a glial

scar that inhibits axonal and neuronal regeneration after injury. Rather, they harbor neural progenitor and stem cell populations allowing them to regenerate entire parts of the nervous system and restore tissue integrity. In the zebrafish retina, cycling neural progenitor cells of the ciliary marginal zone and quiescent resident neural stem cells (also called Müller glial cells) have been involved in the process neuronal regeneration following different types of injury. We have identified an additional cellular source participating in the regeneration of neurons in the zebrafish retina after ablation of retinal ganglion cells. Before injury, these cells express molecular markers of neural crest cells identity, such as sox10, foxd3 or pdgfrb, while after neuronal ablation they also express proneural factors like ascl1a and olig2. Combining genetic ablation of neurons with photoconversion or Cre/Lox dependent genetic lineage tracing of sox10-expressing cells, and cell proliferation analysis, we demonstrated that these cells can differentiate into post-mitotic retinal neurons in the ganglion cell layer (GCL) in the absence of cell proliferation. This work reveals an unexpected cellular mechanism of transdifferentiation, dependent on a neural crest-like cell population, participating in the process of neuronal regeneration in the zebrafish retina. The discovery of this plastic cell population could potentially open new strategies to promote the formation of neurons in the mammalian retina.

Disclosures: R. Madelaine: None.

Poster

PSTR054: Molecular Mechanisms of Axon Regeneration

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR054.16/A42

Topic: D.03. Stroke

Support: JSPS KAKENHIgrant 22H03188

Title: Repeated intravenous mesenchymal stem cell infusions enhance functional recovery beyond single mesenchymal stem cell infusion after acute cerebral ischemia in rats

Authors: *T. YOKOYAMA[1], M. SASAKI[1], H. NAGAHAMA[1], Y. KATAOKA[1], M. NAKAZAKI[1], R. UKAI[1], S. OKA[1], J. D. KOCSIS[2], O. HONMOU[1];
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Abstract: A single intravenous infusion of bone-marrow-derived mesenchymal stem cells (MSCs) has been shown to improve behavioral function in both acute and chronic rat stroke models. However, recent randomized clinical trials of a single MSC infusion for acute cerebral stroke have shown conflicting outcomes. Therefore, an additional therapeutic approach may be necessary to achieve greater functional improvement in stroke therapy. This study investigated whether repeated infusions of MSCs result in greater functional outcomes compared with a single infusion of MSC for the acute phase of cerebral ischemia. Permanent middle cerebral artery occlusion (MCAO) was induced in rats using the intraluminal thread method. After

MCAO induction, we evaluate stroke volume using in vivo T2-weighted MR images (MRI). Thereafter, we compared four MSC regimens: (i) vehicle only, no cells on day 3 (Vehicle); (ii) one standard-dose infusion on day 3 (MSC-1); (iii) one high-dose infusion on day 3 (HD-MSC); and (iv) three standard-dose infusions on days 3, 10, 17 (MSC-3). We examined behavioral function and stroke volume every 7 days from day 3 to day 45 after MCAO induction. On day 45, the rats were perfused transcardially, and their brains were collected. The thickness of the corpus callosum (CC) was measured using Nissl staining. Ex vivo MRI was conducted for the evaluation of the area of the CC and interhemispheric connections within the CC using diffusion tensor imaging (DTI). The greatest motor recovery and the largest increase in CC thickness and area were observed in the MSC-3 group among all groups. Neural tracts within the CC were the most pronounced in the MSC-3 group. These anatomical changes showed a positive relationship with improved behavioral function 45 days after MCAO induction. Furthermore, a positive relationship was found among these anatomical changes. Repeated triple MSC infusion showed greater functional improvement and histological changes in the CC. These results indicate that multiple systemic infusions of MSCs are more effective than a single infusion of MSCs in the acute phase of MCAO, promoting neuroprotection and inducing neural plasticity.

Disclosures: T. Yokoyama: None. **M. Sasaki:** None. **H. Nagahama:** None. **Y. Kataoka:** None. **M. Nakazaki:** None. **R. Ukai:** None. **S. Oka:** None. **J.D. Kocsis:** None. **O. Honmou:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Program #/Poster #: PSTR055.01/A43

Topic: A.06. Developmental Disorders

Support: NIAAA F32 AA029866
NIAAA R01 AA028406

Title: Prenatal alcohol and cannabinoid co-exposure produces sex-specific changes in opioidergic gene expression in the medial prefrontal cortex that are associated with drug-seeking and anxiety-like behaviors.

Authors: *K. RAY[1], S. ROUZER[1], R. C. MIRANDA[2];
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Abstract: The simultaneous use of alcohol and cannabinoids is prevalent among young adults, yet the question of how prenatal co-exposure influences offspring health outcomes is currently underexplored. Limited studies of co-exposure have uncovered synergistic effects on offspring behavioral symptoms compared to single-drug exposures, including behaviors functionally associated with the endogenous opioid system. Two genes of interest include OPRM1 and

PENK, which mediate reward evaluation and anxiety behaviors. In this investigation, we aim to uncover the effects of co-exposure on opioidergic gene expression along the corticostriatal circuit and determine how changes in gene expression correlate with drug-seeking and anxiety-like behaviors.
Pregnant C57B1/6J mice were divided into four treatment groups: drug-naive, alcohol exposed, cannabinoid exposed, or co-exposed, with daily treatments between Gestational Days 12-15. Mice were given an intraperitoneal injection of cannabinoid agonist CP-55940 (750 μg/kg) or vehicle, then transferred to vapor chambers for 30 minutes of 95% ethanol or room air inhalation. Once offspring reached middle age, tissue samples from the dorsomedial striatum (DMS) and medial prefrontal cortex (mPFC) were collected for RNA isolation and sequencing. The relative gene transcript abundance of OPRM1 and PENK was then assessed between exposure groups and sexes, and compared to previously assessed behaviors.
In the mPFC, co-exposure increased PENK expression compared to single-drug exposures in females, while the opposite was seen in males. Moreover, co-exposure reduced OPRM1 expression in the mPFC of females compared to all other exposures. In males, co-exposure increased OPRM1 expression in the mPFC compared to alcohol exposure alone. In within-group comparisons of gene expression and behaviors, alcohol consumption was positively correlated with OPRM1 levels in the mPFC and negatively correlated with PENK levels in the DMS of non-cannabinoid-exposed offspring. A positive correlation was also found between PENK expression in the mPFC and anxiety-like behaviors in co-exposed mice.
Prenatal co-exposure induced distinct, sex-specific changes in PENK and OPRM1 expression in the adult mPFC compared to single-drug exposures and drug-naive controls. Correlations between OPRM1 and PENK in the mPFC and ethanol self-administration suggest that there may be a modulatory relationship between these genes that is disrupted by prenatal drug exposures. In future investigations, we will examine how prenatal co-exposure affects opioid signaling pathways and how this may contribute to the observed behavioral phenotypes.

Disclosures: K. Ray: None. **S. Rouzer:** None. **R.C. Miranda:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Program #/Poster #: PSTR055.02/A44

Topic: A.06. Developmental Disorders

Support: Faneca 66 Foundation
Celebrate hope foundation
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Title: Seizure threshold and BBB in R183Q GNAQ Sturge-Weber Syndrome murine model

Authors: *N. TRUVER, A. M. COMI, C. R. SOLOMON;
Kennedy Krieger Inst., Baltimore, MD

Abstract: Sturge-Weber Syndrome (SWS) is a rare, congenital, neurovascular disorder resulting from a somatic R183Q GNAQ mutation, enriched in endothelial cells. Contrast-enhanced brain MRI demonstrates abnormal, and enhancing leptomeningeal, cortical and subcortical blood vessels. Seizures occur in 90% of infants with SWS before the age of 2, which can worsen cerebrovascular perfusion, brain injury, and cognitive impairments. Kainate (KA), used to stimulate seizure activity, is an excitatory kainate receptor agonist highly expressed in the CA3 region in neuronal cells. We previously demonstrated impaired BBB in mice expressing endothelial mutant human R183Q GNAQ (tetO-*GNAQ**R183Q X *Tie2-rtTA/TRE*-β*Gal*); we hypothesized that these mice would demonstrate increased seizure susceptibility early in life. Additionally, we aimed to assess BBB permeability and general health characteristics after weaning. P15 mice from transgenic litters were given either a 2.0 or 2.5mg/kg KA dose i.p. Mice were then placed in a temperature and humidity-controlled environment and observed for 2.5 hours. Scoring of seizure activity occurred at 5-minute intervals for 150 minutes, blinded to genotype according to a previously published modified Racine score. Mice were perfused at P17. P42 mice were injected intracardially with Evan's Blue and subsequently perfused to assess BBB permeability. Vessel morphology was analyzed with ImageJ and AngioTool by staining sectioned tissue with endothelial marker Tie2. Mutant mice expressing human R183Q GNAQ mutation after 2.5mg/kg KA dose demonstrated worse seizure and higher lethality littermate control. More mutant mice (n=11/12) had tonic-clonic seizure activity at the 2.5mg/kg KA dose than littermate control mice (n=3/7; $p<0.05$); mutant mice also had significantly higher mortality (n=11/12 versus 1/7; $p<0.005$). After 2.0mg/kg KA dose there is a trend for worse seizure severity over time in mutant mice ($\mu=0.73$, SEM=0.22) compared to control ($\mu=0.25$, SEM=0.05) (p=0.059 RMANOVA). At P42 mutant male mice demonstrated decreased body weight (n=15 $\mu=18.8$ versus n=15 $\mu=20.8$; $p<0.005$) and decreased body length (n=15 $\mu=7.8$cm versus n=15 $\mu=8.1$cm; $p<0.05$) compared to littermate male controls; however, the BBB did not demonstrate impairment at this later timepoint. Tissues studies are ongoing. Endothelial R183Q GNAQ mutation may worsen seizure-related outcomes early in life. After weaning, BBB impairments in mutant mice resolve although impairments in growth persist in males. Ongoing and planned studies with brain tissue immunohistochemistry and neuroimaging aim to understand the mechanisms underlying these results.

Disclosures: N. Truver: None. **A.M. Comi:** None. **C.R. Solomon:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Topic: A.06. Developmental Disorders

Support: NIH Grant P20GM139762
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Edna Ittner Pediatric Research support fund

Title: Examining the effects of prenatal alcohol exposure on auditory processing in mouse models of fetal alcohol spectrum disorders

Authors: S. HUNTWORK[1], K. GRAHAM[1], S. ASGHARI[1], P. LEBESSE[1], ***P. RAGUNATHAN**[2];
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Abstract: Exposure to alcohol during pregnancy produces fetal alcohol spectrum disorders (FASD) that are associated with sensory and cognitive deficits. Individuals with FASD have impaired auditory processing and frequently exhibit atypical auditory behaviors. To assess the effects of prenatal alcohol exposure (PAE) on the auditory system, we utilized two alcohol consumption paradigms, maternal voluntary alcohol consumption throughout gestation and a daily limited access ethanol intake model (Drinking in the Dark, DID). We have previously shown that mice prenatally exposed to alcohol (voluntary drinking model, chronic low-moderate PAE) exhibit auditory brainstem response (ABR) abnormalities. In this study, we performed longitudinal investigation (4-week, 7-week and 12-week) of ABR in mice prenatally exposed to alcohol using the DID model (binge-like PAE). We found that PAE females exhibited elevated ABR thresholds at 4 weeks that normalized at 7 weeks. Although PAE males exhibited no significant differences in the hearing thresholds at the 4-week timepoint, elevated thresholds were observed at 7 weeks. Analysis of ABR waves at the 4- and 7-week timepoints showed altered wave latencies in PAE males and females. PAE males were found to exhibit reduced ABR wave amplitudes and elevated amplitude ratios. To examine the impact of PAE on the sensitivity to auditory stimulation, we are currently examining the effects of PAE (chronic low-moderate PAE and binge-like PAE models) on acoustic startle reactivity and short-term habituation of the startle response in 5- to 6-week-old mice. Gap-prepulse inhibition of the acoustic startle response is also been currently performed to examine temporal processing acuity in mice prenatally exposed to the chronic low-moderate exposure model. The goal of our study is to examine the effects of exposure to alcohol during fetal development on the functionality of the auditory pathway.

Disclosures: S. Huntwork: None. **K. Graham:** None. **S. Asghari:** None. **P. Lebesse:** None. **P. Ragunathan:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR055.04/A46

Topic: A.06. Developmental Disorders

Title: Histoarchitecture and immunohistochemical Studies on the Effect of Guts Probiotics Supplementation on Lead-induced Hypothalamic-Pituitary-Gonadal Axis Toxicity using male Wistar Rats

Authors: *A. G. OBAOYE;
Anat., Babcock University, Nigeria, Ogun, Nigeria

Abstract: Lead exposure disrupts the hypothalamic-pituitary-gonadal (HPG) axis, leading to reproductive toxicity. The brain is the center of control for endocrine hormones responsible for reproduction and dysfunction in this center is crucial. Aim: This study investigated the protective effects of gut probiotics supplementation on lead-induced HPG axis toxicity in male Wistar rats. Materials and Method: Ethical approval was obtained from the Research and Ethnics Committee of the University for this study. A total of forty-nine rats, grouped into seven (7) groups; Control (A), Lead only low dose(B), Lead + Probiotics Treatment (C) Probiotics treatment only (D), Lead high dose only (E), Lead high dose + probiotics treatment (F), Probiotics high dose (G) were exposed to lead and/or probiotics, and histoarchitecture and immunohistochemical analyses were performed on reproductive tissues. Results: Oxidative stress, neurotransmitters and inflammatory markers showed that lead exposure altered HPG axis function, while probiotics supplementation mitigated these effects. Conclusion: The findings suggest that gut probiotics may be a potential therapeutic strategy for reducing lead-induced reproductive toxicity.

Disclosures: A.G. Obaoye: None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR055.05/A47

Topic: A.06. Developmental Disorders

Title: Hesperidin Rescues Maternal Separation-Induced Neurobehavioral Deficits Through Coordinated Modulation of Oxidative Stress, Neuroinflammation, and Neurotransmitter Systems

Authors: *V. SINGH[1], M. SINGH[2];
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Abstract: Maternal separation (MS) is a validated early-life stress model that induces long-term neurobehavioral impairments through oxidative stress, neuroinflammation, HPA axis dysregulation, and neurotransmitter dysfunction. This study investigated the neuroprotective potential of hesperidin (HES), a bioactive citrus flavonoid, in a rat MS paradigm. Wistar rat pups underwent MS (3 h/day, postnatal days 2-14) followed by chronic HES treatment (40 and 80 mg/kg/day, p.o.) from weaning through adulthood. Behavioral assessments revealed MS-induced anxiety-like behavior in the elevated plus maze (reduced open arm time), depressive-like behavior in the forced swim test (increased immobility time), and spatial memory deficits in the Morris water maze (prolonged escape latency). These behavioral impairments were accompanied by multifaceted biochemical disturbances including elevated oxidative stress markers, increased pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, NF-κB), HPA axis hyperactivity (elevated

corticosterone), and neurotransmitter imbalances (reduced acetylcholine, serotonin and dopamine with increased acetylcholinesterase activity). HES treatment reversed behavioral deficits and normalized biochemical parameters, demonstrating comprehensive neuroprotection. Notably, HES simultaneously restored redox balance, attenuated neuroinflammation, regulated HPA axis function, and stabilized cholinergic and monoaminergic neurotransmission. These findings highlight HES as a unique multi-target therapeutic agent capable of addressing the complex pathophysiology of early-life stress, with particular translational relevance given its natural origin, established safety profile, and blood-brain barrier permeability. The coordinated improvement across behavioral, neuroendocrine, inflammatory, and neurotransmitter systems positions HES as a promising candidate for preventing stress-related neurodevelopmental disorders.

Disclosures: V. Singh: None. **M. Singh:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Topic: A.06. Developmental Disorders

Support: European Union's Horizon 2020 Framework Programme for Research and Innovation 'Brain Involvement iN Dystrophinopathies (BIND) #847826
Strategic project #23427 of the Association Française contre les Myopathies (AFM, France)

Title: Emotional dysregulation as a key driver of social behavior deficits in a Mouse model of Duchenne Muscular Dystrophy

Authors: *L. H. CESCHI[1], R. MIRANDA[2], C. VAILLEND[1], S. GRANON[1];
[1]Paris Saclay Inst. of Neurosciences, Saclay, France; [2]Complutense Univ. of Madrid, Madrid, Spain

Abstract: Rationale: Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular syndrome caused by mutations in the DMD gene that lead to the loss of one to several brain dystrophin proteins. DMD is frequently associated with intellectual disability, behavioral and neuropsychiatric disorders, but the core endophenotypes underlying these comorbid neurobehavioral deficits are not well defined. Our study investigates how the loss of brain dystrophins affects emotional reactivity and social behavior in a mouse model of DMD. Methods: Using a longitudinal behavioral test battery, we assessed emotional reactivity and social behavior in *mdx52* mice (WT=12, *mdx52*=13) lacking two brain dystrophins (as in 50% of patients). Anxiety was evaluated in the elevated plus maze and light/dark choice tests, stress reactivity with unconditioned fear responses. Social behavior and socially-induced vocalizations were analyzed in various contexts of interactions between *mdx52* males and WT males or

females. We then explored the relationships between emotional and social parameters with multivariate factor analyses. Results: *Mdx52* mice displayed enhanced anxiety and stress responses, and context-dependent alterations of social behavior, notably reduced dominance toward WT females and increased submission during encounters with WT males. This was associated with altered ultrasonic vocalization production and reduced social competitive dominance when grouped for home-cage automated behavioral analyses. Factor analyses revealed correlations between social behavior and emotional reactivity, interfering with dominance-related behaviors. Notably, the relationship between emotional reactivity and social behavior differed between genotypes: in WT mice, fear and anxiety were associated with hierarchical rank, whereas in *mdx52* mice, these emotional parameters were directly associated with social interactions. Conclusion: Together, our findings suggest that the cumulative loss of multiple brain dystrophins leads to context-specific disruptions in social behavior, largely driven by heightened emotional reactivity. This further implements our knowledge of the main endophenotype underlying behavioral disturbances in DMD and provides novel outcome measures for future preclinical studies.

Disclosures: L.H. Ceschi: None. **R. Miranda:** None. **C. Vaillend:** None. **S. Granon:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Topic: A.06. Developmental Disorders

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P50-AA022534

Title: Unsupervised Phenotyping Reveals Disrupted Neural Firing Characteristics in the Anterior Thalamus and Surrounding Brain Regions Following Third-Trimester Equivalent Alcohol Exposure in Mice

Authors: *M. MORNINGSTAR[1,2], G. ACOSTA[3], C. F. VALENZUELA[2], B. J. CLARK[3], D. N. LINSENBARDT[2];
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Abstract: Acute binge-like third-trimester-equivalent alcohol exposure (TTAE) causes apoptosic neurodegeneration in brain regions necessary for spatial learning and memory, such as the anterior thalamus (AT), which encodes context-relevant information, including head direction. While we are beginning to understand the behavioral consequences of this exposure, the neural

correlates of spatial cognition deficits are underexplored. Thus, we recorded a mixture of neurons from the AT and surrounding brain regions in mice with TTAE while they freely moved within a controlled environment. To model acute binge-like TTAE, C57BL/6J mice received 2 injections of 2.5 g/kg alcohol (or saline) on post-natal day (PND) 7. Subjects were then left undisturbed until the day of surgery as adults (>PND 60), when they were implanted with silicon or multi-wire arrays. Mice were placed in a circular 40 cm diameter arena under dim red light with 2 LED cues on the walls that rotated on a pseudo-random basis while electrophysiological data was recorded. Following data preprocessing, spike and waveform features were extracted from each putative single spiking unit. These features were reduced utilizing uniform manifold approximation and projection (UMAP). Following dimensionality reduction, we used agglomerative hierarchical clustering to find populations of neurons with similar features. Following this, we compared each feature based on treatment and found the features most important to disambiguate TTAE's impact on neural activity. TTAE was associated with decreases in mean firing rate, peak firing rate, rebound index, and tail decay constant, increases in alpha, peak to trough time, and repolarization time, and bidirectional differences as a function of neuronal subtype in burst index and rebound index. This suggests that TTAE produces long-lasting and fundamental differences in spiking features that can be observed in vivo and are amenable to intervention. Together, this dataset provides further clarifying criteria that can be utilized to diagnose and treat FASD.

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Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Program #/Poster #: PSTR055.08/A50

Topic: A.06. Developmental Disorders

Support: NIH intramural grant ZIA DK043304-25

Title: Developing a disease model of a clinically-relevant pathogenic GNB5 mutation via cytosine base editing

Authors: *J. SEMSEDDIN[1], H. MCNABB[1], J. ZHANG[1], W. F. SIMONDS[2];
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Abstract: Homozygous or compound heterozygous mutations of *GNB5* have been associated with intellectual developmental disorder with cardiac arrhythmia (IDDCA) syndrome in humans. *GNB5* encodes Gβ5, a divergent isoform of the G protein β essential for normal neuronal development. We previously demonstrated that *Gnb5* heterozygosity enhances the formation of beta-amyloid plaques and neurofibrillary tangles in Alzheimer's Disease (AD) model mice. Given that *Gnb5* heterozygosity may have profound implications in human disease, we are

utilizing CRISPR/Cas9-derived base editing (BE) to develop a mouse model of a pathogenic missense mutation in *Gnb5* to study its phenotypic effects. Through the precise conversion of a target DNA base without double-stranded breakage, base editing offers great potential for modeling and even treating various genetic diseases. As a proof-of-concept, we first introduced an early stop codon in *Gnb5* using the standard SpCas9 in Neuro-2A cell line and verified BE efficacy through fluorescence imaging, Sanger sequencing, and restriction enzyme digest analyses. The results confirmed that the base editor successfully carried out the expected C-to-T conversion in the restriction enzyme sequence within the region of interest targeted by our single guide RNA (sgRNA). In order to expand the range of editable sites and introduce the clinically-relevant *Gnb5* Gly215Glu missense mutation in Neuro-2A cells, we used cytosine BE with NGN and PAM-less SpCas9 variants, namely SpG and SpRY, along with sgRNAs predicted to have high BE efficiency (>50%) and low off-target scores. After enrichment by FACS sorting and validation of editing activity in cell culture, we will transduce the BE complex into mice to generate an IDDCA mouse model, study developmental milestones, and separately examine whether such a heterozygous *Gnb5* pathogenic missense allele can increase neuropathology in AD mice comparably to a heterozygous *Gnb5* null allele. Establishing a successful disease model with an optimized base editing system may enable the study of human pathogenic mutations and aid in identifying potential molecular targets for future therapies.

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Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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JST-CREST

Title: Remote memory deficits in mouse models of neuropsychiatric disorders with immature dentate gyrus phenotype

Authors: *H. SHOJI[1], H. HAGIHARA[1], I. A. GRAEF[2], G. R. CRABTREE[3], F. HUANG[4], P. W. FRANKLAND[5], T. MIYAKAWA[1];
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Abstract: The hippocampal dentate gyrus (DG) is a critical brain region that contributes to recent and remote memory. Granule cells within this region, in which adult neurogenesis occurs,

undergo dynamic and reversible maturation via genetic and environmental factors during adulthood. A pseudo-immature state of DG granule cells, called immature DG (iDG), has been observed in the adult mice of certain mutant strains, which are considered animal models of neuropsychiatric and neurodegenerative disorders, such as intellectual disability, schizophrenia, autism, and Alzheimer's disease. The iDG phenotype is proposed as one of the characteristic features of some types of the disorders. However, the association between the iDG phenotype and recent and remote memories in the mouse models remains unclear. We assessed spatial memory in the Barnes circular maze task in five mutant mouse models of the disorders with the iDG phenotype, including Camk2a heterozygous knockout (HET KO), forebrain-specific Calcineurin conditional KO (cKO), Neurogranin KO, and Hivep2 (Schnurri-2) KO, and hAPP-J20 transgenic mice. Camk2a HET KO mice and J20 mice spent less time around the target than their wild-type control mice in the memory retention tests one day and four weeks after the last training session, with the memory impairments being more pronounced at the remote delay. Calcineurin cKO, Neurogranin KO, and Schnurri-2 KO mice showed no significant differences in the time spent around the target from wild-type mice in the retention test 1 day after the training session, but those mutants spent less time around the target than their wild-type mice in the retest conducted four weeks later, which exhibited selective deficits in remote memory. These results indicated that mouse models of neuropsychiatric and neurodegenerative disorders exhibiting the iDG phenotype demonstrate a common behavioral characteristic of remote spatial memory deficits, suggesting the potential involvement of the pseudo-immature state of DG granule cells in remote memory dysfunction.

Disclosures: H. Shoji: None. **H. Hagihara:** None. **I.A. Graef:** None. **G.R. Crabtree:** None. **F. Huang:** None. **P.W. Frankland:** None. **T. Miyakawa:** None.

Poster

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The Hartwell Foundation

Title: The role of the dyslexia-associated gene KIAA0319 in regulating intrinsic excitability in the zebra finch (Taeniopygia guttata) auditory cortex

Authors: *T. M. BYRON[1], C. D. DEPPMANN[2], C. MELIZA[2];
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Abstract: Developmental dyslexia is a common learning disorder that affects approximately 10% of the population and results in a marked decrease in phoneme processing, suggesting an

underlying deficit in auditory processing. Dyslexia is associated with loss-of-function mutations in *KIAA0319* (Galaburda et al., 2006), but the function of this gene remains poorly understood. Reduced *KIAA0319* expression in rodents results in electrophysiological and behavioral auditory deficits (Centanni et al., 2014). We hypothesize that *KIAA0319* may play a role in experience-dependent plasticity of intrinsic neuronal dynamics during phonetic learning. Like humans, zebra finches grow up in complex acoustical environments and learn how to recognize conspecific vocalizations during an early sensitive period, which makes them a useful model to test this hypothesis. We characterized how expression of *KIAA0319* in the zebra finch auditory pallium (cortex) varies across developmental stages and acoustical environments using in situ hybridization and immunofluorescence. Preliminary results indicate experience-dependent *KIAA0319* expression around 30 days post hatch, which corresponds to the peak of song memorization in males and a period of major functional plasticity (Moseley and Meliza, 2025). To test for a causal role in this plasticity, we developed adeno-associated viruses (AAVs) carrying short-hairpin ribonucleic acid (shRNA) constructs targeting *KIAA0319*. This approach allows us to investigate whether the developmental knockdown of *KIAA0319* impairs song learning, alters intrinsic plasticity, and modulates the expression of other proteins implicated in neuronal plasticity.

Disclosures: T.M. Byron: None. **C.D. Deppmann:** None. **C. Meliza:** None.

Poster

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NIH U19 NS104649
NIH R01 NS123648
NIH R01 NS106983

Title: Cortical network and metabolic alterations in HCN1-linked developmental epileptic encephalopathy

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Abstract: Developmental epileptic encephalopathies (DEEs) are severe pediatric disorders often caused by mutations in genes coding for voltage-gated ion channels, including hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which regulate neuronal excitability via the hyperpolarization-activated current (I_h). A clinically relevant HCN1 variant (p.G391D, or HCN1-GD) has been modeled in mice, which exhibit spontaneous generalized tonic-clonic seizures as well as persistent behavioral comorbidities. Previous studies have monitored seizure activity and behavior separately. However, little is known about how large-scale neural network activity is altered in DEEs in awake behaving animals, particularly outside of seizure events. Using wide-field optical mapping (WFOM), we simultaneously recorded cortical neural activity, hemodynamics, and behavior in awake, head-fixed HCN1-GD mice expressing a neuronal calcium indicator during both whisker-stimulus evoked and spontaneous behavior where mice did not exhibit seizures. Motor activity and pupil diameter were tracked to assess arousal level and shifts in behavioral state. We found that HCN1-GD animals displayed aberrant patterns of spontaneous behavior, including restlessness, and abnormal bursts of calcium activity. Initial results indicate altered neurovascular responses during stimulus-evoked and spontaneous behaviors, suggesting possible altered metabolic function. After in-vivo imaging, histology revealed varying extents of hippocampal degeneration (mesial temporal sclerosis) as seen in other mouse models with limbic seizures.
Our ongoing studies are characterizing the relationship among alterations in behavior, cortex-wide neuronal dynamics, and neurovascular coupling in HCN1-GD mice. Together, these findings may offer a window into how DEEs disrupt brain function and behavior outside periods of seizure activity.

Disclosures: K. Swayze: None. **B. Santoro:** None. **N. Rungratsameetaweemana:** None. **S.A. Siegelbaum:** None. **E.M.C. Hillman:** Other; Leica.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR055.12/A54

Topic: A.06. Developmental Disorders

Support: University of Otago PhD scholarship to SE Kohe

Title: In a novel rat model of ADHD-like hyperactivity and impulsivity after delayed reward, both behaviors correlate with changes in forebrain monoamines

Authors: *D. E. OORSCHOT[1], E. GOWING[2], Y. ZHENG[3], I. C. KOKAY[4], D. R. GRATTAN[2], P. LIU[2], S. SEO[2], S. E. KOHE[5];
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Abstract: Children born extremely prematurely can experience repeated hypoxic injury to the brain. They can also develop hyperactivity that is attention deficit hyperactivity disorder (ADHD)-like, as well as impulsivity. Males are more vulnerable. In a new Sprague-Dawley rat model of repeated hypoxic brain injury during the equivalent of extreme prematurity, ADHD-like hyperactivity and impulsivity after delayed reward were observed in males at 3 months-of-age (Int. J. Mol. Sci. 2023, 24, 11252). ADHD-like behaviours were not observed in the control repeated normoxic rats. Based on the literature, we hypothesized for the same cohort of animals that: (i) the observed ADHD-like hyperactivity may be correlated with changes in the cerebral metabolism of dopamine and/or noradrenaline and (ii) the observed ADHD-like impulsivity may be correlated with changes in the cerebral metabolism of serotonin. These monoamines and their metabolites were investigated in the caudate-putamen and prefrontal cortex using high performance liquid chromatography, and precise brain dissection, at 7-months-of-age. Using data from the 11 hypoxic and 8 normoxic rats, increased ADHD-like hyperactivity, and increased ADHD-like impulsivity, both had a highly significant correlation with a decreased absolute amount of the dopamine metabolite 3-4-dihydroxyphenylacetic acid (DOPAC), and a decreased DOPAC/dopamine ratio from absolute data, in the right prefrontal cortex. Increased ADHD-like impulsivity also had a highly significant correlation with a decreased concentration of DOPAC, and with a decreased DOPAC/dopamine ratio from concentration data, in the right prefrontal cortex. Increased ADHD-like hyperactivity, and increased ADHD-like impulsivity, both had a significant correlation with a increased absolute amount of noradrenaline in the left prefrontal cortex. Increased ADHD-like hyperactivity was significantly correlated with an increased concentration of serotonin in the right posterior caudate-putamen. These results indicate that ADHD-like hyperactivity and ADHD-like impulsivity correlate with decreased cerebral metabolism of dopamine in the right prefrontal cortex and with increased cerebral metabolism of noradrenaline in the left prefrontal cortex. These changes support the hypothesis. In contrast, ADHD-like hyperactivity, but not ADHD-like impulsivity, correlates with increased serotonin metabolism in the right posterior caudate-putamen. The mainly right-sided changes are consistent with ADHD being a right-brain disorder. Overall, the data provide strong evidence that ADHD-like behaviors correlate with changes in forebrain monoamines.

Disclosures: D.E. Oorschot: None. **E. Gowing:** None. **Y. Zheng:** None. **I.C. Kokay:** None. **D.R. Grattan:** None. **P. Liu:** None. **S. Seo:** None. **S.E. Kohe:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Program #/Poster #: PSTR055.13/A55

Topic: A.06. Developmental Disorders

Support: CMCR Grant P64-07-001

Title: Prenatal Cannabidiol Exposure Influences Early Motor Development in Rats

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Abstract: Cannabis is the most commonly used illicit drug among pregnant women. Consumption of cannabidiol (CBD), a non-psychoactive component of cannabis, has increased in popularity among pregnant women, with over 20% reporting use within the past 30 days. This popularity is due to the perception that CBD is safe to use during pregnancy, despite a limited literature suggesting that CBD may affect the developing fetus. Thus, the purpose of this study was to examine the effects of prenatal CBD exposure on early motor development. Pregnant rats were given 50 or 0 mg/kg of CBD daily during gestational days 5-20 via a honey and cookie dough vehicle. Then, on postnatal days (PD) 2-12, offspring were tested on various early motor reflexes, including righting, grasping, and geotaxis reflexes, in which success and latency (time) were recorded. On PD 12-20, grip strength and hindlimb coordination were measured in the offspring by recording whether they could hang from an elevated bar for 30 s and get their hindlimbs onto the bar. Prenatal CBD exposure led to advancements in reflex development that were sex-dependent. Advancements in motor reflexes were seen in CBD-exposed male subjects, whereas CBD-exposed females had advancements in grip strength and hindlimb coordination. Thus, prenatal CBD exposure may alter the developmental trajectory of motor development. Interestingly, when motor function was tested in adolescent subjects, there were mild motor impairments among CBD-exposed subjects. These results suggest that CBD use during pregnancy may pose risks for the developing fetus. Supported by CMCR P64-07-001.

Disclosures: M. Rusnak: None. **D. Miramontes:** None. **I. Fleischer:** None. **J.D. Thomas:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Program #/Poster #: PSTR055.14/A56

Topic: A.06. Developmental Disorders

Support: CAPES
CNPQ
FAPERJ

Title: Equilibrative Nucleoside Transporter Type 1 modulation impacts locomotive and risk-related behavioral parameters in an animal model of Attention-Deficit/Hyperactivity Disorder

Authors: *S. C. VALLADÃO[1], A. DOS SANTOS-RODRIGUES[2], P. PANDOLFO[1,2]; [1]Grad. Program in Biomed. Sciences: Physiol. and Pharmacol., [2]Inst. of Biol., Federal Fluminense Univ., Niteroi, Brazil

Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental condition mainly caused by dopaminergic imbalance in specific brain regions, which can be normalized by adenosinergic signaling. Extracellular adenosine levels are crucial for this signaling and can be regulated by nucleoside transporters, such as the equilibrative nucleoside transporter type 1 (ENT1). Our study aims to investigate ENT1 levels in key brain regions and to explore the impact of ENT1 blockade on behavioral tasks using a validated animal model of ADHD. All procedures were approved by the Institutional Ethics Committee on Animal Use under protocol #7023070523. The brain frontal cortex, striatum, and hippocampus from adult male and female Wistar Kyoto (WKY) and Spontaneously Hypertensive Rats (SHR) were collected and processed for synaptosomal and total membrane fractions. Protein density of each sample was quantified. Hippocampal synaptosomal ENT1 levels were higher in adult female SHRs (1.51±0.23, n=5) than in males (0.89±0.12, n=5; p=0.0358), as well as in striatal total membranes (males: 0.56±0.08, n=5; females: 0.53±0.07, n=5; p=0.0434). There were no differences in frontocortical and hippocampal total membrane, or in cortical and striatal synaptosomal ENT1 levels, when comparing sexes and strains. Furthermore, adolescent male and female rats received either saline or NBTI 15mg/kg intraperitoneal injections and underwent open field (OF) and elevated plus maze (EPM) tests for the assessment of locomotor, emotional, and risk-related behavioral parameters. In the OF, adolescent female SHRs were hyperlocomotive (32.40±1.71, n=4) in comparison with male SHRs (28.58±1.09, n=5; p=0.0268), as well as in the EPM (males: 15.80±0.90, p=0.0184; females: 18.41±1.36, p=0.0011). Treatment reduced locomotion in male (10.95±1.92, p=0.0002, n=6), but not female (26.41±2.76, n=5) SHRs, in the OF and EPM (males: 7.17±1.00, p=0.004; females: 16.79±1.70). Male (50.71±4.25, p=0.0271) and female (44.23±3.92, p=0.0041) SHRs spent more time in the center of the OF, reduced only in males after treatment (40.70±5.11; p=0.0091). Male SHRs spent less time in the center of the EPM (50.71±4.25, p=0.0108), not affected by NBTI. GraphPad Prism software was used for statistical analyses (two-way ANOVA, Tukey's post hoc comparisons). Therefore, based on our study, higher levels of ENT1 in striatal synaptosomes and hippocampal total membranes, especially in females, may contribute to ADHD symptoms. Further research is necessary to determine whether ENT1 levels play a significant role in the behavioral variations between younger male and female rats in this animal model of ADHD.

Disclosures: S.C. Valladão: None. **A. dos Santos-Rodrigues:** None. **P. Pandolfo:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR055.15/A57

Topic: A.06. Developmental Disorders

Support: UVA Brain Institute
UVA Program in Fundamental Neuroscience
UVA Department of Psychology

Title: The impact of postnatal immune challenge on premature birth outcomes

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Abstract: Preterm birth is associated with increased risk of atypical neurodevelopment and cognitive deficits, including autism spectrum disorder (ASD). Furthermore, preterm infants admitted to the neonatal intensive care unit (NICU) have an increased risk of developing sepsis. As postnatal inflammation alone is associated with ASD, even in term-born children, it is unclear whether prematurity itself, or prematurity paired with neonatal infection predisposes infants to atypical neurodevelopment. Our study isolates the effect of prematurity and neonatal infection alone ("single-hit") and in combination ("double-hit") to investigate the etiology of atypical neurodevelopment in preterm populations. We used an established mifepristone (MFP) model of prematurity, paired with lipopolysaccharide (LPS) injection at postnatal day (PND) 3 in mice to mimic neonatal sepsis. Premature mice are born a day earlier than term counterparts. We used early communication and behavioral assays to investigate outcomes of prematurity and postnatal inflammation. At PND7, we found an increase in separation-induced ultrasonic vocalizations (USVs) of term mice administered LPS, but no significant differences in frequency or length. No significant differences were observed at PND14 or 21. Double-hit mice aged PND21-35 displayed increased ambulation in open-field assays, and reared significantly more. In a novel object recognition (NOR) task, all mice regardless of prematurity or inflammation preferred novelty to familiarity. In 3-Chamber Tasks (3CT), all mice significantly preferred social to nonsocial interactions, and novel social interactions to familiar interactions. Altogether, our results demonstrate that prematurity in combination with early neonatal infection ("double-hit") has lasting effects on ambulation in adolescence, but does not significantly impact novelty preference or social behaviors. Further contextualization of innate behaviors throughout adulthood in single and double-hit mice are necessary to elucidate potential long-lasting compound effects of inflammation and prematurity.

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Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Program #/Poster #: PSTR055.16/A58

Topic: A.06. Developmental Disorders

Support: NIMH 1RM1MH138313-01

Title: Scalable Behavioral and Molecular Phenotyping of Genes Associated with Neuropsychiatric Disorders

Authors: *R. CHASE[1], S. E. MALONEY[2], H. OVIEDO[3], A. V. KRAVITZ[2], H. GABEL[3], J. D. DOUGHERTY[4], E. JONES[1], K. B. MCCULLOUGH[1], S. CHATURVEDI[1], R. MELLOR[3], R.

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Abstract: In recent years, advancements in the field of genetics have led to the discovery of numerous gene variants associated with neuropsychiatric disorders (NPDs), like autism, intellectual disabliity, and schizophrenia. While the association is clear, less is known about how these genes impact disease physiology, especially in the brain. As part of the SSPsyGene Consortium, we at the Washington University Scalable Mouse Assay Center (WU-SMAC), aim to comprehensively evaluate the phenotypic consequences of NPD-risk gene loss on mammalian brain development, organization, and function. Using a standardized high-throughput set of behavioral assays and molecular transcriptomics techniques, WU-SMAC aims to evaluate the role of over 100 of these NPD risk genes in mouse models. To evaluate risk gene loss behaviorally, we utilize automated home-cage monitoring of activity, learning behavior, and cognitive flexibility. Subsequently, individual mouse and social behaviors are investigated using high-content, machine-learning driven behavioral analyses. Finally, we assess molecular, cellular, and system level consequences of risk gene loss by performing both spatial transcriptomics and single nucleus RNA sequencing. Completion of this systematic pipeline across over 100 mutant mouse lines will result in large amounts of data that will provide invaluable insights into individual risk genes and allow for direct comparisons between mouse lines. Along with data, this effort has begun and will continue to produce many key resources for the overall NPD research community. Upon introducing this work, we will be able to share data with, incorporate feedback from, and further expand the field of scalable, systematic NPD research.

Disclosures: R. Chase: None. **S.E. Maloney:** None. **H. Oviedo:** None. **A.V. Kravitz:** None. **H. Gabel:** None. **J.D. Dougherty:** None. **E. Jones:** None. **K.B. McCullough:** None. **S. Chaturvedi:** None. **R. Mellor:** None. **R. D'Souza:** None. **T. Law:** None. **M. Nemera:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Topic: A.06. Developmental Disorders

Support: FAPERJ E-26/201.081/2022
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CNPq 313353/2021-2
CAPES Código de Financiamento 001

Title: Maternal organophosphorus pesticides (OPPs) exposure and the association with sex-dependent behavioral changes in the adult offspring

Authors: *A. C. MANHAES[1], V. H. S. D. DUARTE[2], P. LISBOA[3];
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Abstract: OPPs are the most used worldwide and have been associated with changes in the central nervous system, inducing cognitive dysfunction. We hypothesize that maternal exposure to glyphosate and acephate during pregnancy and lactation, pesticides that are the among the ones most used in Brazil, may alter offspring behavior in adulthood. On the 7th day of gestation, pregnant rats were exposed by gavage to one of the following solutions: vehicle (filtered water; Control group; CON), glyphosate (5 mg/kg of body mass, BM-GLY5 or 0.5 mg/kg BM-GLY0.5) or acephate (4.5mg/kg bw-ACE4.5 or 0.45mg/kg bw-ACE0.45). At adulthood (120-days-old; n=8/group), we evaluated male and female offspring's body mass gain, food intake, food preference test (by offering a standard (SD) and a high-fat (HF) diets for 30 min and 12h), the anxiety-like behavior (using the elevated plus maze-EPM), and locomotor activity (using the open field-OF). Ethics approval: CEUA/UERJ 004/2020. All groups of both sexes, except for ACE0.45 group, had low birth weight when compared to CON ($p < 0.05$ for all significant pairwise comparisons). At adulthood, there was no difference in body mass among groups. However, except for ACE0.45 females, all animals showed reduced food intake when compared to controls during the study ($p < 0.05$ for all significant pairwise comparisons). Regarding the feeding experiment, ACE4.5 females showed high preference for the HF diet after 30 min (SD+2.9x vs. CON; $p < 0.05$). In the EPM, ACE4.5 males showed an increase in total entries (+59%, $p < 0,05$; higher locomotor activity). GLY5 females showed a reduction in total entries (-29%, $p < 0,05$; lower locomotor activity) and ACE4.5 females showed a reduction in the percentage of open arms entries (-30%, $p < 0.05$; anxiety-like behavior).Maternal OPPs exposure had adverse effects on biometric and behavioral parameters of the adult progeny, depending on the dose, pesticide and sex of the offspring. We suggest that maternal OPPs can increase risk for cognitive dysfunctions leading to health consequences in the long term.

Disclosures: A.C. Manhaes: None. **V.H.S.D. Duarte:** None. **P. Lisboa:** None.

Poster

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Topic: A.06. Developmental Disorders

Support: CONAHCYT doctoral scholarship 814093 awarded to BMR
Cuerpo Académico de Neurofisiología (UV-CA-333)
Proyecto SIREI (No. DGI: 242922024175)

Title: Prenatal stress disrupts early behavior without altering hippocampal NKCC1 or KCC2 expression or seizure susceptibility

Authors: *B. MARTÍNEZ-ROJAS[1], V. VENTURA[2], C. MORGADO-VALLE[3], L. BELTRAN-PARRAZAL[3], L. D. CHI-CASTAÑEDA[3], F. CHENA-BECERRA[4], C. ZAVALA-TECUAPETLA[5], L. LOPEZ-MERAZ[3];
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Abstract: Prenatal stress has both short- and long-term deleterious effects on the developing brain, including increased susceptibility to epileptic seizures. One proposed mechanism involves altered expression of the chloride cotransporters NKCC1 and KCC2, which regulate intracellular chloride levels and influence the excitatory or inhibitory action of GABA via $GABA_A$ receptors. Such alterations may disrupt GABAergic signaling and promote seizure activity during development. In this study, we investigated the effects of prenatal restraint stress on neurological development, hippocampal NKCC1 and KCC2 expression, and seizure susceptibility in rat pups. Pregnant Wistar rats were subjected to restraint stress (RS, n = 15) twice daily from gestational day (GD) 12 to GD20. Control dams (C, n = 15) remained undisturbed. Offspring underwent a behavioral test battery from postnatal day (P) 3 to P21 (C females n = 14, RS females n = 16; C males n = 16, RS males n = 14), including the surface righting test, open field test, negative geotaxis test, and bar holding test. NKCC1 and KCC2 expression in the hippocampus were assessed at P7, P14, and P21 in both sexes (females n = 10, males n = 10, per group at each age). At P7, pentylenetetrazol (a $GABA_A$ receptor antagonist; 100 mg/kg, s.c.) was used to induce seizures in all groups (C females n = 9, RS females n = 11; C males n = 9, RS males n = 12). Prenatally stressed pups showed delayed neurological development, evidenced by a higher failure rate in the negative geotaxis test at P4 (females) and increased latency in the righting reflex (both sexes) from P3 to P7. Prenatal stress did not alter hippocampal NKCC1 or KCC2 expression at any age, nor did it affect seizure incidence, severity, or latency to generalized seizures. Notably, seizure-related mortality was significantly increased in prenatally stressed males compared to controls. Together, these findings suggest that prenatal restraint stress impairs early motor, vestibular, and proprioceptive development in a sex-dependent manner. However, the hippocampal GABAergic system appears resilient to prenatal stress and is unlikely to mediate its effects on seizure vulnerability.

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Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR055.19/A61

Topic: A.06. Developmental Disorders

Title: A New Mouse Model of Hyperglycinemia Encephalopathy: Forebrain-Specific Astrocytic GlyT1 Knockout with Spontaneous Seizures

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Abstract: Background: Nonketotic hyperglycinemia (NKH) is a severe neurometabolic disorder marked by excess glycine in the brain, leading to seizures, cognitive impairments, and sensory abnormalities. Current global GlyT1 knockout models are limited by neonatal lethality and systemic effects, hindering long-term mechanistic and therapeutic studies. **Methods:** We developed a novel forebrain-specific astrocytic GlyT1 knockout mouse (Gfa2-GlyT1 KO) using Cre-lox recombination targeting GFAP-expressing astrocytes. This preserves brainstem GlyT1 expression, enabling postnatal survival and study of glycine dysregulation in forebrain circuits. Gfa2-GlyT1 KO, heterozygous, and wildtype littermates were assessed. (i) Body and brain weights were measured at key developmental stages. (ii) Immunohistochemistry (IHC) confirmed GlyT1 deletion specificity and assessed NMDA and glycine receptor (NMDAR, GlyR) expression in the hippocampus and cortex. (iii) Intrahippocampal EEG electrodes were implanted at P21 for continuous video-EEG monitoring of spontaneous seizures. **Results:** Gfa2-GlyT1 KO mice survive into adulthood and exhibit spontaneous, self-limited seizures and failure to thrive. (i) Gfa2-GlyT1 KO mice showed significantly reduced body weight across the developmental to adult periods. (ii) Seizures typically emerged between postnatal days 25 and 60, with behavioral seizure phenotypes ranging from Racine Scale 1 to 4. Clinical seizures consisted of myoclonic jerks - rapid, short, electric shock-like muscle contractions - accompanied by altered breathing. Seizures can last from several minutes to half an hour and are self-limited, with mice returning to normal ambulation, eating, and drinking behavior without intervention. (iii) IHC data demonstrated the deletion of GlyT1 limited to the forebrain astrocyte, especially in the hippocampus of KO mice, while retaining brain stem GlyT1 expression. The NMDAR and GlyR expressions were also altered in the hippocampus and cortex of Gfa2-GlyT1 KO compared to WT littermates. **Conclusion:** The Gfa2-GlyT1 KO mice have selective loss of GlyT1 expression in the hippocampus and cortex. Targeted forebrain depletion of GlyT1 allowed long-term survival into adulthood and was sufficient to cause epilepsy. This mouse model enables chronic, circuit-specific investigation of hyperglycinemic and epileptic encephalopathy, providing a robust platform for elucidating disease mechanisms and evaluating targeted therapies for developmental and epileptic encephalopathy (DEE) associated with forebrain glycine dysregulation.

Disclosures: N. Ferris: None. **S. Koh:** None. **H. Shen:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

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Topic: A.06. Developmental Disorders

Support: NINDS/NIH R01 RNS107428

Title: Hemizygous rare variants in the coiled-coil domain containing 120 (CCDC120) cause a distinct neurodevelopmental disorder, including intellectual disability, developmental delay, and microcephaly

Authors: *T. AKHTER[1,2], Z. M. AHMED[1,3,4], D. WEGNER[5], E. RAHIKKALA[6], R. RABIN[7], L. URPA[8], T. STEFAN BARAKAT[9], A. FERNÁNDEZ-JAEN[10], A. SHILLINGTON[11], T. BIERHALS[12], S. RIAZUDDIN[13], S. RIAZUDDIN[14,15];
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Abstract: *CCDC120* (OMIM:300947) encodes a coiled-coil domain-containing protein 120, implicated in cytoskeletal remodeling and neurite outgrowth. Through the multi-institutional collaboration and employing the whole-exome sequencing approach, we identified one frameshift and thirteen hemizygous missense variants in *CCDC120* across eighteen affected individuals from fourteen ethnically diverse families, all presenting with a spectrum of neurodevelopmental disorder (NDD) phenotypes. Clinical manifestations included intellectual disability (mild to severe), speech delay, craniofacial dysmorphism, epilepsy, microcephaly, ADHD, skeletal anomalies, hypotonia, motor delays, behavioral abnormalities, and sensory impairments. To elucidate the pathogenic mechanisms underlying *CCDC120*-associated NDD, we employed a multidisciplinary approach including, *in silico* modeling, cellular assays, and animal models. Structural predictions and 3D protein modeling indicated that the missense variants disrupt protein folding and molecular interactions. Functional assays in HEK293 and Neuro2a cells demonstrated significantly reduced mRNA and protein stability for mutant proteins, along with aberrant subcellular localization; mutant proteins accumulated perinuclearly rather than trafficking along neurites to growth cones, as observed with wild-type protein. We further generated a *CCDC120* knockout SH-SY5Y neuroblastoma cell line using CRISPR/Cas9 genome editing to investigate its role in neurogenesis. Preliminary differentiation studies are underway. *In vivo*, morpholino-mediated knockdown of *ccdc120* in *neurod1-GFP* transgenic zebrafish recapitulated key patient phenotypes, including microcephaly, craniofacial skeletal defects, and impaired motor responses to visual and acoustic stimuli. Whole-mount brain imaging revealed reduced brain volume, diminished neuronal populations, and disrupted

cerebellar architecture. Notably, these phenotypes were rescued by co-injection of wild-type human *CCDC120* mRNA but not by transcripts encoding NDD-associated variants, supporting a loss-of-function mechanism. Collectively, our findings establish *CCDC120* as a critical regulator of neurodevelopment and demonstrate that its disruption contributes to a distinct NDD phenotype through loss-of-function mechanisms.

Disclosures: T. Akhter: None. **Z.M. Ahmed:** None. **D. Wegner:** None. **E. Rahikkala:** None. **R. Rabin:** None. **L. Urpa:** None. **T. Stefan Barakat:** None. **A. Fernández-Jaen:** None. **A. Shillington:** None. **T. Bierhals:** None. **S. Riazuddin:** None. **S. Riazuddin:** None.

Poster

PSTR055: Animal Models of Developmental Disorders Other Than Autism I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR055.21/B1

Topic: A.06. Developmental Disorders

Support: NIH GRANT 3R01NS112291-05S1
Myotonic Dystrophy Foundation Pre-doctoral Fellowship

Title: Splicing Dysregulation and Behavioral Deficits in a Novel Neuronal Mouse Model of Myotonic Dystrophy Type 1

Authors: *B. BEKELE[1], J. D. ARBOLEDA[3], J. P. SCHROEDER[5], J. JIANG[2], E. T. WANG[4], G. J. BASSELL[6];
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Abstract: Myotonic dystrophy type 1 (DM1) is a multi-systemic, autosomal dominant disorder caused by CTG repeat expansions in the DMPK gene. In addition to muscle pathology, DM1 affects the central nervous system (CNS), leading to symptoms such as intellectual disability, cognitive impairment, and social behavior deficits. To investigate the developmental and circuit-level consequences of RNA-mediated toxicity in the brain, we generated a neuron-specific DM1 mouse model by crossing CaMKIIα-tTA mice with TREDT960I mice, which carry 960 interrupted CTG repeats in the human DMPK 3′UTR. This Tet-Off system enables inducible expression of toxic RNA selectively in forebrain neurons, including cortical and hippocampal regions critical for learning and social behavior. Using Fluorescent *In-situ* Hybridization (FISH) and immunofluorescence, we observed RNA foci and nuclear sequestration of MBNL1/2 primarily in the cortex and hippocampus. Bulk RNA-sequencing revealed mis-splicing of several neuronal genes, including GABRG2, which encodes the gamma 2 subunit of the $GABA_A$ receptor and GRIN1 gene which encodes the GluN1 subunit of the N-methyl-D-aspartate (NMDA) receptor, both of which have previously been implicated in neurodevelopmental disorders. We also conducted behavioral analysis of mice expressing CUG repeats and observed

deficits in associative learning and memory as well as reduced social recognition. Our findings demonstrate that neuronal expression of toxic RNA disrupts alternative splicing events essential for neurodevelopment and synaptic function. This model provides a valuable tool to study CNS involvement in DM1 and highlights shared molecular and behavioral features with other mouse models of neurodevelopmental disorders.

Disclosures: B. Bekele: None. **J.D. Arboleda:** None. **J.P. Schroeder:** None. **J. Jiang:** None. **E.T. Wang:** None. **G.J. Bassell:** None.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.01/B2

Topic: A.07. Development of Neural Systems

Title: Generation of Human Cerebellar Organoids to Investigate Human-Specific Features of Cerebellar Development

Authors: *N. HOSSEINI[1,2];
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Abstract: The cerebellum has long been associated with motor control, but growing evidence highlights its involvement in humans' higher-order cognitive functions and emotional regulation. Understanding how the human cerebellum develops its unique cellular diversity is crucial for uncovering its full scope of functions and role in physiological and pathological conditions. Comparative studies reveal significant differences between human and murine cerebellar development. In mice, cerebellar neurogenesis originates from two progenitor zones: an inhibitory ventricular zone (VZ) and an excitatory Rhombic Lip, both lacking a subventricular zone (SVZ). In contrast, human cerebellar development is marked by a distinct expansion and compartmentalization of these progenitor zones, each forming its own SVZ containing mitotic basal progenitors. These basal progenitors, characterized by long radial fibers and mitotic radial glial markers, expand within the SVZ and contribute significantly to human-specific cerebellar neurogenesis. Such distinctions are key to understanding species-specific brain development, but limited access to the human brain necessitates alternative models. Human pluripotent stem cell-derived cerebellar organoids (hCerOs) have emerged as a promising in vitro system to interrogate the early stages of development and disease of this human brain region. We have recently established a robust and reproducible protocol for generating cerebellar organoids from human induced pluripotent stem cells (hiPSCs) to model key aspects of human cerebellar development. These organoids give rise to all major cerebellar cell types, including both excitatory and inhibitory neurons, and multiple glial subtypes. Using this protocol, I generated human cerebellar organoids containing basal radial glial cells with morphological and spatial features closely resembling those found in human fetal cerebellar tissue. In parallel, I applied our

optimized methodology to mouse embryonic stem cells (mESCs) to generate mouse cerebellar organoids, in which I also identified basal radial glial cells localized within the ventricular zone. This comparative system provides a unique platform to investigate species-specific aspects of cerebellar development, particularly the role and distribution of basal radial glia. Finally, I describe our ongoing efforts using lineage tracing approaches to map the developmental trajectories of cerebellar progenitors within organoids, focusing on their contributions to inhibitory and excitatory lineages during cerebellar development.

Disclosures: **N. Hosseini:** A. Employment/Salary (full or part-time): Keck school of medicine of USC, Development, Stem cell, and Regenerative medicine, Los Angeles, CA.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.02/B3

Topic: A.07. Development of Neural Systems

Support: NIMH IRP (ZIA MH002984)

Title: Connectivity of the primate-specific thalamic pulvinar underpins postnatal maturation of frontoparietal cortex

Authors: ***J. T. SCOTT**[1,2], A. CACCAVANO[3], M. WALKER[2], K. A. PELKEY[3], J. JONTZ[2], K. MARCHE[2], G. A. VARGISH[3], S. HUNT[3], J. A. BOURNE[2];
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Abstract: Among the largest nuclei of the primate thalamus, the medial pulvinar (PM) coordinates distributed cortical networks for cognitive processing. During visual attention in primates, PM connectivity acts as an indirect pathway for communication between the prefrontal cortex (PFC) and the parietal cortex, enabling flexible control of attentional allocation. Recent evidence suggests that these connections may be developmentally regulated, as PM-PFC connectivity during early life defines outcomes for adult cognitive function. However, whether this impacts function at the network level remains unknown. Here, we explored this question by permanently inactivating PM during early life in the marmoset monkey (*Callithrix jacchus*) and examining its effect on the adult frontoparietal network. We employed MRI-guided stereotaxic surgery to ablate the bilateral PM using excitotoxic NMDA at infancy (P14; n=2 lesion, n=2 control). We then characterized changes in network connectivity during adolescence by performing diffusion MRI at pre-adolescence (6 mo.) and early adulthood (18 mo.). Tractography revealed that a reduction in connectivity between the PM and the frontoparietal cortex following lesions coincided with reduced connectivity between PFC and parietal cortex. To investigate consequences for attention, we then trained marmosets in adulthood (>18 mo.) to

perform a novel visuospatial directed attention task, delivered via touchscreens in the home-cage. Preliminary findings revealed specific differences in behavioral output between control and lesioned animals. Next, we dissected the impact of individual circuit mechanics by focusing on connections between the PM and PFC. From diffusion MRI, diffusivity maps revealed that microstructural changes in PFC grey matter across adolescence were arrested following PM lesion. To explore this further, we examined known targets of PM afferents, parvalbumin-positive interneurons (PV-INs), by surgically injecting AAV carrying the S5E2 enhancer in the adult PFC. Histology revealed immature morphology of PV-INs following PM lesion, including reduced soma size and neurite branching. In addition, slice electrophysiology showed pronounced changes in firing properties, including attenuation of the stereotypical fast-spiking phenotype. These findings demonstrate the long-lasting impact of early PM connectivity in establishing circuits responsible for cortical inhibition, which coincide with correlates of frontoparietal network impairment. This work supports an evolving view of the thalamus, in which spatially distributed connectivity informs cortical development long into postnatal life.

Disclosures: J.T. Scott: None. **A. Caccavano:** None. **M. Walker:** None. **K.A. Pelkey:** None. **J. Jontz:** None. **K. Marche:** None. **G.A. Vargish:** None. **S. Hunt:** None. **J.A. Bourne:** None.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.03/B4

Topic: A.07. Development of Neural Systems

Title: Identifying the molecular footprints of sociability in the developing canine embryo brain

Authors: *O. REVAH[1], A. MARCO[2];
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Abstract: Canis lupus familiaris, the domestic dog, exhibits remarkable genetic and behavioral diversity shaped by evolution alongside humans. Despite extensive behavioral studies, the developmental neurobiology underlying this diversity remains largely unexplored. Here, we introduce a novel approach utilizing primary embryonic dog brain tissue at critical developmental stages to investigate canine neurodevelopment and gene expression dynamics. Employing advanced whole brain 3D imaging, we identified pronounced cortical expansion occurring late in canine gestation. Immunohistochemical analyses revealed abundant outer radial glial cells, a hallmark of prolonged corticogenesis, suggesting developmental parallels between canine and human neocortex formation. Notably, gene expression profiling demonstrated that the canine neocortical transcriptional landscape in late gestation closely resembles that of the human brain at approximately 20 post-conceptual weeks. This approach allowed us to explore similarities and differences between the species. Finally, we show selective and dynamic developmental expression of Williams-Beuren Syndrome -associated genes, recently linked to sociability in dogs, predominantly in deep and superficial prefrontal cortical neurons. Our

findings provide insights into the anatomical and transcriptional landscapes of canine brain development, and may offer new directions for comparative neurobiological studies on the molecular underpinnings of mammalian sociability.

Disclosures: O. Revah: None. **A. Marco:** None.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.04/B5

Topic: A.07. Development of Neural Systems

Title: Developmental mechanisms of neural spacing mediated by DSCAM and ECM interactions

Authors: *K. HIZAWA[1], Y. U. INOUE[2], S. MIYASHITA[2], T. INOUE[2], T. SASAKI[1,3], M. HOSHINO[2], N. ARIMURA[1];
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Abstract: The brain primarily consists of two types of cells: neurons, which form electrical circuits, and non-neuronal glial cells, particularly astrocytes, which integrate into circuits by covering a vast number of synapses in both humans and rodents (Dallérac *et al*., *Nat Rev Neurosci*, 2018). During development, astrocytes migrate, divide, and occupy non-overlapping territories to provide efficient and non-redundant support (Ge *et al*., *Nature*, 2012). Thus, proper astrocyte arrangement is essential for normal brain function. However, how they recognize neurons and spread evenly to achieve circuit-wide coverage remains unclear. Down Syndrome Cell Adhesion Molecule (DSCAM) is a transmembrane protein expressed in neurons and related to regulating intercellular spacing, dendritic self-avoidance, and neuronal migration (Hizawa *et al*, *Neurosci Res*, 2024). Here we report that DSCAM interacts with extracellular matrix (ECM) molecules, mainly secreted by vascular endothelial cells. This result raises the possibility that DSCAM-ECM complexes may repel each other, thereby facilitating the insertion of glial cells between neurons, and contributing to the formation of neural circuits. To examine this, we performed 3D primary cultures involving targeted ECM proteins using brains from embryonic day 15 (E15) mice. *Dscam* knockdown revealed that intercellular neuronal distance was significantly reduced, indicating the function of DSCAM in maintaining appropriate neural spacing. Knockdown of *Dscam*-associated ECM-encoding genes *in vivo* was carried out at E13.5, when the blood-brain barrier is still immature, by using intracerebroventricular injection of gene-silencing agents (Clarke *et al*., *Mol Ther*, 2024). Western blot analysis using E14.5 and E15.5 mouse embryonic brain lysates confirmed the targeted ECM protein reduction. By using this *in vivo* knockdown method, we aim to elucidate the functional roles of DSCAM and an ECM protein in regulating intercellular neural spacing and astrocyte tiling among neurons. Given

that neuronal DSCAM has been implicated in neurodevelopmental disorders such as epilepsy and schizophrenia, and that astrocyte dysfunction is also increasingly recognized as a contributing factor in these pathologies, the interaction between DSCAM and astrocytes may hold important clinical implications for understanding and potentially treating such disorders.

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Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.05/B6

Topic: A.07. Development of Neural Systems

Title: Comparative Analysis of Supplements on Neuronal and Stem cell Culture Applications

Authors: R. JOSEPHSON[1], A. IBRAHIMI[1], P. SHAHI THAKURI[1], R. SCOTT[2], K. LAHA[3], D. KUNINGER[1], *S. SHIN[1];
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Abstract: The B27 supplement, widely used in various cell culture applications including neurons, human and mouse pluripotent stem cells (PSCs), and their differentiation, has led to the development of similar formulations by several vendors. Although these alternatives are known by different names, they aim to allow similar functionalities. This study investigates the effects of these alternative supplements on several cell culture applications, such as mouse and rat neurons, human neural human neural stem cell (NSC) proliferation, and human PSC differentiation. We conducted a comparative analysis to assess the impact of these alternative formulations on cell culture outcomes. Detailed analytical studies were performed to uncover the differences between B27 and other supplements. Our findings help provide critical insights into the variations in cell culture outcomes when using these alternative formulations, independent of the specific vendors. This research enhances our understanding of how supplement affects cell culture applications, thereby aiding in the optimization of cell culture protocols and ensuring reproducibility in experimental studies.

Disclosures: R. Josephson: A. Employment/Salary (full or part-time): Thermofisher Scientific. **A. Ibrahimi:** A. Employment/Salary (full or part-time): Thermofisher Scientific. **P. Shahi Thakuri:** A. Employment/Salary (full or part-time): Thermofisher Scientific. **R. Scott:** A. Employment/Salary (full or part-time): Thermofisher Scientific. **K. Laha:** A. Employment/Salary (full or part-time): Thermofisher Scientific. **D. Kuninger:** A. Employment/Salary (full or part-time): Thermofisher Scientific. **S. Shin:** A. Employment/Salary (full or part-time): Thermofisher Scientific.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.06/B7

Topic: A.07. Development of Neural Systems

Support: Intramural Research Program of NIMH ZIC MH002952-09
Intramural Research Program of NIMH ZIA MH002951-09

Title: Cholinergic control of vocal-emotional behavior in the developing marmoset (Callithrix jacchus)

Authors: *K. CHRISTOPHER[1], I. INGRAM[1], C. HUME[1], H. HARKINS[1], D. MATROV[1], S. BRADLEY[2], Y. CHUDASAMA[1,2];
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Abstract: Cholinergic neurons play an essential role in refining cognitive-emotional components of executive function by interacting with the activity of prefrontal-hippocampal networks. A major population of these neurons originate from the basal forebrain nuclei that lie as a continuum extending from the medial septum, vertical and horizontal diagonal bands of broca through the nucleus basalis of Meynert. During development, acetylcholine modulates neuronal proliferation, differentiation, and synaptic plasticity but its contribution to the development and maturation of cognitive, social and emotional processing remains unknown. In this study we show how early life degeneration of medial septal cholinergic projections in infant marmosets alters the normal development of vocal behavior. We targeted the medial septal-basal forebrain nuclei by injecting ME20.4-IgG-saporin (saporin) in 14-day old neonatal marmosets (n=6). Saporin is an immunotoxin that selectively destroys the p75-positive cholinergic cells. Another group of infant marmosets received sham control surgery (n=6). Several key changes were observed. First, vocal calls such as phee, twitter, trill and tsik were preserved before and after the saporin immunolesion but there were notable changes in the overall structure of the calls. For example, infant specific cry-calls that trigger parental feedback were intact, but their cries were short in duration and low in frequency. Second, at four weeks of age, both, the control and saporin group of animals appeared oblivious to a fear-provoking object; they neither approached nor exhibited defensive reactions to it, and tsik alarm calls were notably absent. By postnatal week 12, some fear-like behaviors emerged in the control group but animals with medial septal cholinergic depletions appeared heightened in their anxiety showing a combination defensive reactions together with alarm tsik calls. Third, the saporin immunolesion altered the animals normal interaction with its parent. Control infants and their parents reciprocated touch behaviors and spent more time in close proximity to each other whereas the saporin group of animals appeared ambivalent in their behavior showing both approach and withdrawal behaviors. Together, these data suggest that the normal development of medial septal-basal forebrain cholinergic projections are integral to the postnatal development of vocal-emotional and social behavior.

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Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

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Program #/Poster #: PSTR056.07/B8

Topic: A.07. Development of Neural Systems

Support: FONDECYT 1231556 (JMC)
FONDECYT 1231685 (CO)
ANID 21240414 PhD Fellowship (IA)

Title: Unveiling the Role of Transglutaminases in Brain Development and Function: The Case of Dopaminergic Neurons

Authors: I. ALMONACID[1], J. ALMONACID[2], A. CHAMORRO[4], R. V. ZÁRATE[5], M. GONZALEZ RAMIRE\[6], C. OLIVA[4], M. E. ANDRES[3], ***J. M. CAMPUSANO**[7];
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Abstract: Transglutaminases (TGs) are a family of enzymes characterized by their calcium-dependent transamidation activity. In higher mammals, 8 catalytically active TG members have been reported, each one encoded by different genes. Due to its high expression in the CNS, many studies have focused on the contribution of TG2 to brain formation and functioning. Intriguingly, TG2 KO mice show no major neurodevelopmental abnormalities, and no evident alterations are detected in their CNS. This might be explained by compensation by the other 5 TG members also expressed in mammalian CNS. Thus, it is evident that simpler animal models are needed to study the contribution of TGs to brain formation and operation. In that regard, *Drosophila melanogaster* has only one TG-encoding gene (dTg). Regrettably, little is known of the roles of this protein in fly brain development or physiology. Here we sought to investigate the contribution of dTG in the development of *Drosophila melanogaster* CNS. Using two different genetic tools to interrupt *dTg* gene expression, we observed marked morphological alterations in the fly mushroom body (MB), an important associative area in *Drosophila* brain. Interestingly, we also detected a reduction in dopaminergic neurons in larval and adult stages. Cultured *Drosophila* dopaminergic neurons from *dTg* knock-down (KD) larval brains exhibited reduced neurite branching when compared to control cultures, supporting a contribution of dTG to brain and dopamine neuron development. Furthermore, dTG-deficient animals showed reduced fly locomotion and strong asocial-like phenotype, two behavioral phenotypes associated to the dopaminergic system. Interestingly, expressing human hTG6 under the control of the *Drosophila* endogenous *dTg* promoter rescued the social behavior while failing to restore motor output.

Consistent with these findings, transcriptomics experiments revealed changes in the expression of several genes associated with neurogenesis and neurodevelopment in dTG-deficient animals as compared to controls. Our data support that TGs contribute to brain formation and operation.

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Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

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Program #/Poster #: PSTR056.08/B9

Topic: A.07. Development of Neural Systems

Support: NIH Grant U01MH130962-03

Title: Tracing Cellular Lifespans in the Brain through Optimal Transport

Authors: *Y. MARGHI[1], C. VAN VELTHOVEN[2], U. SÜMBÜL[2];
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Abstract: Understanding development, aging and the transition between them at single-cell resolution is critical for deciphering the molecular logic that shapes cellular lifespans in the brain. During development, processes such as lineage specification, proliferative expansion, and spatial organization establish the adult cellular landscape[1,2]. Conversely, aging introduces widespread gene expression changes across neuronal and non-neuronal populations, reduces immature neuronal types, and dampens the expression of genes involved in signaling and structure[3]. Despite the availability of millions of single-cell transcriptomic profiles, tracing the continuous evolution of individual cells across time points remains experimentally challenging due to the lack of longitudinal profiling. Here, we present a novel computational framework that leverages optimal transport to infer cellular trajectories from scRNA-seq snapshots of the developing and aging mouse brain. While traditional optimal transport approaches often rely on sample-to-sample transportation[4] (consider each cell as an individual point mass), such methods scale poorly and perform sub-optimally in the presence of large datasets. In contrast, our approach models cellular populations at each time point as mixtures of Gaussian components, where each component represents a subpopulation of cells. This formulation allows us to compute efficient, parametric optimal transport maps between these population-level distributions, capturing underlying structure, and providing a scalable, continuous landscape of cell-type transitions across time. By applying this framework, we can investigate molecular mechanisms in development and aging, identify key cell types and genes driving differentiation and temporal transitions, and infer the temporal changes of cell states throughout the lifespan. Additionally, this approach can reveal both shared and distinct patterns of cellular transitions between these two opposing, yet complementary processes in the brain. Ultimately, our method

advances understanding of neurodevelopment and aging by connecting static transcriptomic snapshots into time-resolved trajectories, demonstrating conserved and divergent features of brain biology.
1. La Manno G, et al., “Molecular architecture of the developing mouse brain.” Nature, 2021.
2. Kaplan HS, et al., “Sensory input, sex and function shape hypothalamic cell type development.” Nature, 2025.
3. Jin K, et al., “Brain-wide cell-type specific transcriptomic signatures of healthy ageing in mice.” Nature, 2025.
4. Klein D, et al., “Mapping cells through time and space with moscot.” Nature, 2025.

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Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

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Topic: A.07. Development of Neural Systems

Support: Ann S. Bowers Women's Brain Health Initiative
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Miguel Servet Type II research contract (CPII21/00016)

Title: A digital twin of the pregnant brain: modeling structural brain changes during human gestation

Authors: *A. H. MYERS[1], S. KUSHNER[2], M. MARTÍNEZ-GARCÍA[3], D. MARTIN DE BLAS[4], M. PATERNINA-DIE[4], C. BALLESTEROS[4], E. G. JACOBS[5], S. CARMONA[6], N. MIOLANE[2];
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Abstract: Pregnancy induces one of the most substantial hormonal changes in the human body, with levels of ovarian hormones—particularly estrogen and progesterone—increasing by 100 to 1,000 fold during gestation. These hormonal shifts have pronounced effects on the brain, but the patterns and timing of brain structure changes during pregnancy remain poorly understood due to the scarcity of longitudinal pregnancy brain imaging data.
This data gap is beginning to be addressed by large-scale efforts such as the Women's Brain

Health Initiative. A group working within this initiative published the first longitudinal MRI dataset tracking a woman's brain across the full course of pregnancy. To address this knowledge gap, we present the first digital twin of the female brain during pregnancy—a computational model that predicts how brain structure changes as a function of gestational age or circulating hormone levels. Specifically, we model these changes using a regression framework. We first train the regression model, where the input variable X represents either gestational week or a vector of hormone levels (e.g., estrogen, progesterone), and the output Y is a brain mesh composed of vertices and faces. Then, the trained regression model is able to provide brain mesh predictions for any configuration of hormone levels or gestation week. This approach allows us to generate predicted brain morphologies at arbitrary points during gestation, without requiring longitudinal MRI data from individual subjects.
Our model focuses on regions known to be highly sensitive to sex hormones, particularly the hippocampus and surrounding structures. Prior longitudinal studies have reported measurable reductions in hippocampal volume during pregnancy, believed to be driven by elevated estrogen and progesterone levels.
By offering predicted brain meshes from either gestational week or hormone concentrations, our digital twin provides a non-invasive baseline estimate of brain structure across pregnancy. This enables researchers to visualize the expected trajectory of brain changes and opens new possibilities for integrating brain modeling with clinical or behavioral data.
This model represents an important step forward in studying the neurobiology of pregnancy. It lays the groundwork for future investigations into how pregnancy-related brain changes may relate to cognition, affective processing, or mental health, and holds potential for future applications in obstetrics, psychiatry, and women's health research.
Adele Myers and Sarah Kushner are co-first authors of this abstract.

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Poster

PSTR056: Principles of Building a Brain

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Program #/Poster #: PSTR056.10/Web Only

Topic: A.07. Development of Neural Systems

Support: Pratiksha Trust

Title: Second Trimester Human Cortical Development Scales According to the Universal Power Law for Gyrification

Authors: *S. DHIVAHAR[1], R. VERMA[1], V. KARTHICK[1], G. KUMARAN[1], R. RANGASAMI[2], H. KUMAR[3,4], B. MOTA[5], M. SIVAPRAKASAM[1];
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Abstract: The multifaceted development of the human cerebral cortex must be systematically characterized to understand brain maturation and the mechanisms of cortical folding (CF)—one of the most prominent structural changes during development. Based on a universal power-law relating total cortical surface area (*At*), average cortical thickness (*T*), and exposed surface area (*Ae*), linearizing this relationship yields three independent variables—*K* (folding/mechanical stress), *S* (shape factor), and *I* (isometric scaling)—providing a concise, quantitative framework for describing cortical morphology across species and developmental stages. We tested this universal scaling law during the second trimester of human cortical growth using N=40 non-pathological postmortem fetal specimens (13-25 gestational weeks). In-skull gradient T1-weighted MRI datasets (3T GE Signa Architect), after appropriate preprocessing with a well-established pipeline, were used to model CF. The developing cortical plate—representing future adult gray matter—was manually segmented with quality control and reconstructed in 3D to generate reproducible binary masks. Left and right hemispheres were analyzed independently as surface meshes to extract *At*, *Ae* (approximated via a convex hull), and cortical volume (*V*). *T* was estimated as the ratio *V/At* and increased from 0.45 to 1.16 mm with gestational age ($R^2 \approx 0.66$, $p < 1e\text{-}9$). As expected, the gyrification index (*At/Ae*) also showed an upward trend, though with a weaker fit ($R^2 \approx 0.3$, $p < 0.001$). *A*t, *V*, and hemispheric volumes increased consistently with age ($R^2 > 0.89$, $p < 1e\text{-}18$). We observed a strong relationship between $At \times \sqrt{T}$ and *Ae* ($R^2 > 0.98$, $p < 1e\text{-}30$), as predicted by the universal scaling law. Among the derived variables, *K* did not show a clear trend with gestational age ($R^2 < 0.005$, $p > 0.5$), reflecting high inter-individual variability and a non-uniform CF rate. *S* showed a weak but increasing trend ($R^2 \approx 0.37$, $p < 5e\text{-}05$), possibly reflecting heterogeneous cortical shaping influenced by complex cellular and genetic mechanisms. Meanwhile, *I* increased linearly with age ($R^2 \approx 0.93$, $p < 1e\text{-}21$), indicating steady cortical size expansion. These findings validate the universal scaling law during the second trimester, showing that despite cortical complexity, CF follows predictable patterns under structural constraints like increasing thickness and the evolving white matter architecture. Normative *K*, *S*, and *I* trajectories from 13-25 gestational weeks offer a developmental benchmark, capturing key aspects of shape and size, and aid in mapping cortical maturation from early gestation to adulthood.

Disclosures: S. Dhivahar: None. **R. Verma:** None. **V. Karthick:** None. **G. Kumaran:** None. **R. Rangasami:** None. **H. Kumar:** None. **B. Mota:** None. **M. Sivaprakasam:** None.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.11/Web Only

Topic: A.07. Development of Neural Systems

Support: Prime Minister's Research Fellowship, Government of India
The Pratiksha Trust

Title: Developmental Trajectory of the Hippocampal Formation Across the 2nd Trimester Fetal Brain

Authors: *R. RAVEENDRAN[1], M. BOTA[2], M. SIVAPRAKASAM[1], H. KUMAR[3], R. VERMA[2];
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Abstract: The human hippocampal formation (HCF) undergoes complex structural maturation during fetal development, establishing the scaffold for mnemonic and spatial functions. Despite its importance, the normative development of the HCF during the second trimester of human fetal development is not very well understood. We describe here the developmental trajectory of the HCF across the second trimester (14-24 gestational weeks; GW). Five specimens were fixed in 4% paraformaldehyde, serially cryosectioned in the sagittal plane at 20 µm, and Nissl-stained. Digitized sections were manually annotated using an in-house developed tool to delineate HCF parts, to create detailed cytoarchitectural maps at each GW. Using these maps, we provide a systematic and detailed description of the dentate gyrus (DG), the CA fields, and the subicular complex during the second trimester of gestation, latero-medially, and dorso-ventrally. Firstly, we observe the progressive trilaminar organization in the DG, characterized by the migration of cells from the fimbrial/dentate neuroepithelial sources, leading to the formation and compaction of the granule cell layer by 17 GW, and the subsequent formation of the polymorphic layer by 20 GW. This results in the DG adopting a distinct U shape by 24 GW, coinciding with the morphological development of the hippocampal fissure. In parallel, the CA region exhibits limited laminar differentiation at 14 GW; by 16 GW, the emergence of a putative pyramidal layer permits initial cytoarchitectural distinction among CA1-CA3 subfields. CA1 and CA2 mature earlier than CA3, reflecting regional neurogenic gradients, while the putative CA4 is observed by 24 GW. Although laminar architecture becomes more distinct with age, most cells remain rounded and immature until 24 GW, when pyramidal neurons appear in the deepest cortical plate layers of CA1 and CA2.The subicular complex develops into presubiculum, subiculum, and parasubiculum, each with distinct cytoarchitectural features, as early as 14 GW. Notably, we observe the development of the characteristic cell islands of the presubiculum and its lamina dissecans as early as 16 GW. Across all regions, we document a ventro-dorsal gradient of maturation, with the ventral hippocampal limb developed prominently by the 24 GW. These findings offer a detailed spatio-temporal atlas of hippocampal morphogenesis, and lay the groundwork for interpreting developmental disruptions underlying neurodevelopmental disorders.

Disclosures: R. Raveendran: None. **M. Bota:** None. **M. Sivaprakasam:** None. **H. Kumar:** None. **R. Verma:** None.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.12/B11

Topic: A.07. Development of Neural Systems

Support: Office of the Principal Scientific Adviser, Government of India
The Pratiksha Trust

Title: Quantifying human white matter development of the second trimester using deep UV-C Block face imaging

Authors: *J. JAYAKUMAR[1], A. KALLADAYIL SHIBU[2], S. KARTHIK[2], J. JOSEPH[2], R. VERMA[3], M. SIVAPRAKASAM[2];
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Abstract: Understanding the development of white matter fiber tracts in the human fetal brain is an important step in understanding brain development. Our current understanding of this important step in brain development has been limited due to the poor resolution of methods such as MRI and also the variations in the tissue structure of the brain during development, including lack of myelination of the fibers during the early fetal brain development. We have utilized a novel technique using Block face imaging (BFI) under deep ultraviolet (UV-C) illumination to quantify the growth and features of the developing white matter tracts of the second trimester fetal brain (14-25 gestational weeks). Our technique enhances relative contrast between the gray matter and the developing fiber tracts, thus allowing the quantification of the tracts. In addition, we show the organization of these tracts across the whole brain at a better resolution than MRI (60- 80um per pixel). We have segmented using a Medical Segment Anything model (MedSAM) guided machine learning technique, the major fiber tracts including corpus callosum, cingulum bundle, internal capsule, external capsule, cerebral peduncle, cerebellar peduncle, longitudinal fasciculi, Uncinate Fasciculus**,** anterior and posterior commissures. We also show their relative development in 3D across the second trimester. Our findings not only establish that the UV-C BFI as a powerful modality for the detailed analysis of fetal WM development but also provide a valuable resource for the quantification of white matter fiber tract development in humans.

Disclosures: J. Jayakumar: None. **A. Kalladayil Shibu:** None. **S. Karthik:** None. **J. Joseph:** None. **R. Verma:** None. **M. Sivaprakasam:** None.

Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.13/B12

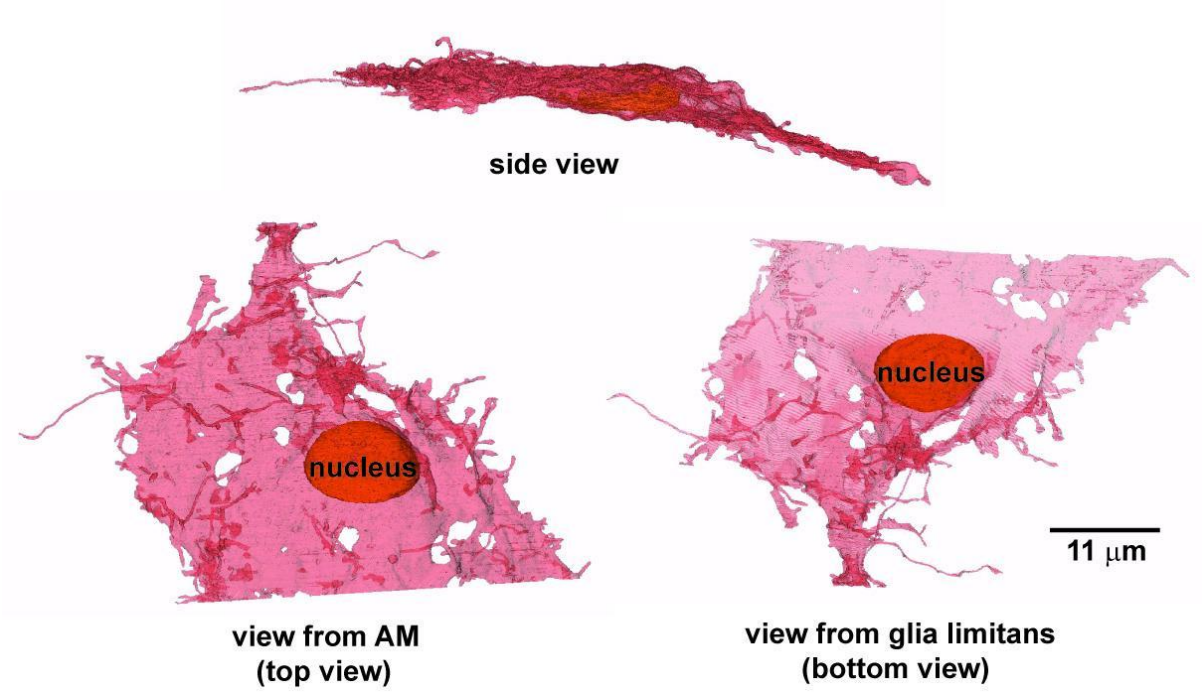
Topic: A.07. Development of Neural Systems

Support: Region Normandie (Chaire d'Excellence)
National Institute for Physiological Sciences Japan

Title: Tiny threads, big connections: 3D mapping of leptomeningeal cell interactions during early brain development

Authors: *H. KOMURO[1], A. DEBONNE[1], A. LEBON[1], M. BÉNARD[1], D. SCHAPMAN[1], C. CHAMOT[1], A. ARABO[1], S. BERNARD[1], P. CHAN[1], S. LOOPRASERTKUL[2,3], N. ONO[2,3], L. GALAS[1];
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Abstract: Mammalian brains are covered by the leptomeninges, which consist of the pia mater (PM) and arachnoid mater (AM). Anatomically and molecularly distinct cell types are found in the leptomeninges, but intercellular interactions between leptomeningeal cells are poorly understood. In this study, we examined nano-scale connections between leptomeningeal cells by analyzing detailed three-dimensional structures of the cells, which were created by using serial block-face scanning electron microscopy (SBF-SEM). Ten postnatal 2-day-old and postnatal 4-day-old mice (CD-1, both sexes) were used. Animal experiments were conducted in accordance with the French guide for the care and use of laboratory animals and the European Community Council Directive. Mice were perfused with 2.5% glutaraldehyde and 4% paraformaldehyde. Cerebella with leptomeninges were dissected out from the other parts of the brain, stained with heavy metals, and embedded in resin. To obtain a series of EM photos, we used SBF-SEM (Gatan 3view/Zeiss ΣIGMA/VP & JEOL IT800) and array tomography SEM system (Zeiss ATLAS5). Serial images sequences of cerebellar leptomeningeal cells were generated at 50 nm steps, providing image stacks >37 μm deep and 33x33 μm wide at a resolution of 5.5 nm per pixel. Serial images of EM were processed, measured with ImageJ using FIJI plugins, including TrakEM2, and used to construct 3D-images of cells. 3D-images of pia fibroblasts in P4 mouse are presented in a figure. At this developmental stage, pia fibroblasts have several large holes in their extended paper-like thin cell bodies. Also, pia fibroblasts extend many tiny processes (50-300 μm in diameter) in three different directions [upward to subarachnoid space (SAS), downward to subpial space (SPS), and laterally within PM], and form nano-scale connections with arachnoid fibroblasts in AM, macrophages in SAS, microglia in SPS, and neighboring pia fibroblasts in PM. These results suggest that cerebellar leptomeningeal cells may interact with each other by means of nano-scale connections during early development.



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Poster

PSTR056: Principles of Building a Brain

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.14/B13

Topic: A.07. Development of Neural Systems

Support: JST SPRING program JPMJSP2123

Title: Reelin-dependent migration of Purkinje cells drives cerebellar foliation

Authors: *R. UCHI[1], T. KASAI[1,2], T. HONDA[1,3], Y. HIROTA[1], K. NAKAJIMA[1];
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Abstract: How the gyri of the mammalian brain are formed has been of great interest, but the mechanisms remain incompletely understood. Because cerebellar foliation is commonly observed across many species, the basic mechanisms of cerebellar foliation are thought to be shared among various species and might provide insights into the general principles of gyrus formation. Reelin and its downstream cytoplasmic adaptor protein, DAB1, play crucial roles in neuronal migration and positioning during brain development. Although it is known that loss of

Reelin or DAB1 leads to extremely decreased volume and foliation of the cerebellum, the underlying mechanisms remain largely unknown. In this study, we investigated the role of Reelin signaling in cerebellar development using *Dab1* conditional knockout (cKO) mice. Injection of adeno-associated virus (AAV) expressing Cre under the Purkinje cell (PC)-specific L7 promoter into the lateral ventricles of *Dab1*-floxed mice at P1 resulted in PCs with abnormal positioning at P8, being embedded in the internal granular layer in lobules VI, VII, and X, associated with shallowed fissure between lobules VI and VII. In normal development, PCs begin to disperse toward the cerebellar surface around E15, form a multilayered structure around birth and finally form a monolayer by P4. Since we found that monolayer formation occurs later in lobules VI, VII, and X than in other lobules, and that most PCs in these regions remain multilayered at P1, DAB1 was considered necessary for the PC monolayer formation. Consistent with this idea, injection of Cre-expressing AAV at P4 (after the completion of monolayer formation) had no effect on PC positioning and cerebellar foliation. Furthermore, injection of AAV at E15 resulted in the infection of certain clusters of PCs and abnormal positioning and foliation in those regions. These observations suggest that DAB1 is required for PC migration up to the formation of the monolayer, but afterward, DAB1 does not contribute to PC positioning or foliation. They also clearly demonstrate a spatial relationship: normal foliation occurs in regions where PCs migrate properly, whereas foliation is disrupted where their migration fails. Finally, BrdU pulse-labeling experiments revealed that proliferation of granule cell precursors (GCPs) decreased in *Dab1* cKO, suggesting that PC abnormal positioning may impair proliferation of GCPs. Taken together, our results suggest that Reelin signaling regulates PC positioning and cerebellar foliation by modulating GCP proliferation.

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Poster

PSTR056: Principles of Building a Brain

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR056.15/B14

Topic: A.07. Development of Neural Systems

Support: U01 Arlotta

Title: A Spatial Transcriptomic Atlas of the Developing Mouse Brain: Enabling the Study of Cortical GABAergic Interneuron Migration

Authors: *R. MATHIEU, M. KUNST, L. CHING, J. QUON, D. MCMILLEN, N. MARTIN, J. NAGRA, P. OLSEN, N. VALERA CUEVAS, M. VANNESS, J. CAMPOS, A. RUIZ, J. ARIZA TORRES, S. BARTA, M. REDING, E. LIANG, C. HUANG, N. URIBE, O. HELBACK, Y. GAO, M. HEWITT, S. C. SEEMAN, S. DANIEL, L. NG, C. M. PAGAN, C. VAN VELTHOVEN, Z. YAO, J. WATERS, H. ZENG;
Allen Inst. for Brain Sci., Seattle, WA

Abstract: The mammalian brain comprises millions to billions of cells, organized into diverse cell types with distinct spatial distributions and functional properties. In the adult mouse brain, a comprehensive, high-resolution transcriptomic atlas of the adult mouse brain has been generated by integrating whole-brain-scale single-cell RNA sequencing and MERFISH datasets, as shown in the Allen Cell Types Database. This approach revealed a strong correspondence between transcriptomic identity and spatial localization. The remarkable diversity of cell types in the adult brain arises through sequential gene expression changes during neural stem cell maturation and differentiation. While hundreds of molecularly defined cell types have been characterized in the adult brain, their developmental origins and the regulatory programs guiding their formation remain largely undefined. To address this, we applied a set of validated technology platforms to generate a spatially resolved, single-cell atlas of the developing mouse brain with high temporal resolution. Using MERFISH, we captured the spatial distribution of transcriptomically defined cell types. By leveraging state-of-the-art computational methods, both in-house and externally developed, we successfully constructed whole-brain atlases across key time points. This developmental atlas enables the study of biological processes such as the migration of GABAergic interneurons into the isocortex, an essential step in cortical circuit formation. However, the molecular mechanisms guiding their precise positioning remain poorly understood. To visualize this process across all developmental stages, we employed a unified coordinate system, allowing consistent spatial comparisons through time. Ultimately, this framework aims to identify cell type-specific molecular signals guiding the migration and integration of cortical GABAergic neuron.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.01/B15

Topic: A.10. Schizophrenia

Support: MRC Grant: MR/X010910/1

Title: Schizophrenia risk gene Grin2A: Effects of heterozygous knockout on behavioural flexibility and spatial representation.

Authors: *M. H. SABEC[1], L. ALBERIO[1], M. CLAYDON[1], S. BAJABA[1], M. C. ASHBY[1], D. M. KULLMANN[2], E. S. ROBINSON[1], J. R. MELLOR[1];

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Abstract: Schizophrenia is a neurodevelopmental disorder characterized by severe psychiatric symptoms and is associated with a significant burden on affected individuals, as well as their clinical and emotional support networks. Vulnerability to schizophrenia arises through a complex architecture of genetic risk factors, including the cumulative effects of common genetic variants which individually contribute small risk, and ultra rare high-penetrance mutations which confer larger genetic risk. Such ultra rare, high-risk variants were recently identified in 10 genes through the schizophrenia exome sequencing meta-analysis (SCHEMA) consortium, including a loss-of-function protein-truncation mutation in the *Grin2A* gene which encodes the developmentally regulated GluN2A subunit of the NMDA receptor.

We propose that loss-of-function mutations in *Grin2A* disrupt dendritic Ca^{2+} signalling, leading to impaired synaptic plasticity. This disruption is expected to perturb the encoding and flexible remapping of spatial representations by hippocampal place cell populations.

To investigate this hypothesis, we performed two-photon calcium imaging in heterozygous *Grin2A* knockout and wild-type mice engaged in virtual reality (VR) behavioural tasks. Mice were injected with a calcium indicator (AAV-hSyn-GCaMP8m) targeted to the dorsal CA1 region of the hippocampus and implanted with a 3 mm diameter deep cranial window. After recovery, mice were trained to run head-fixed along a linear VR track to obtain a sucrose water reward delivered at a fixed location within the environment.

Learning of the reward location and track familiarity was assessed through behavioural measures, including running speed and anticipatory licking near the reward site. Concurrent two-photon imaging (920 nm excitation, 30 Hz frame rate, 457μm^2) was used to monitor neural activity in response to environmental novelty and reward location shifts. Spatially tuned place cells were identified and analysed to assess stability, remapping, and global changes in spatial representation across familiar environments, altered reward conditions, and novel contexts, respectively.

Bridging the behavioural and neural consequences of high-risk genetic variants will provide deeper insight into the mechanisms underlying the psychiatric and cognitive deficits observed in individuals with schizophrenia. This understanding may inform the development of more precise and personalized therapeutic interventions.

Disclosures: M.H. Sabec: None. **L. Alberio:** None. **M. Claydon:** None. **S. Bajaba:** None. **M.C. Ashby:** None. **D.M. Kullmann:** None. **E.S. Robinson:** None. **J.R. Mellor:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.02/B16

Topic: A.10. Schizophrenia

Support: MRC grant MR/X010910/1

Title: Schizophrenia risk gene Grin2a: Effects of heterozygous knockout on cellular properties, synaptic integration and plasticity.

Authors: *M. D. B. CLAYDON[1], M. H. SABEC[2], L. ALBERIO[1], S. BAJABA[3], M. C. ASHBY[4], D. M. KULLMANN[5], E. S. ROBINSON[1], J. R. MELLOR[6];
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Abstract: Schizophrenia is a complex and severe neurodevelopmental disorder with multiple neurotransmitter systems and brain regions implicated in the variety of symptoms experienced by patients. Alterations to glutamatergic signalling, in particular hypofunction of the NMDA receptor, have been proposed to be central to the development of neuropathological changes. Recent whole-exome sequencing data aggregated ultra-rare coding variants to identify genes significantly associated with schizophrenia. A number of genes associated with synaptic function, including Grin2a, which codes for the GluN2A subunit of the NMDA receptor, were identified.There is evidence for hippocampal involvement in the development of schizophrenia, and disruptions to plasticity in the hippocampus are proposed to contribute to the cognitive impairments associated with schizophrenia. In the present study, we generated male and female Grin2a+/- mice and utilized hippocampal slice preparations to characterize electrophysiological and synaptic alterations in heterozygous knockouts compared to their wild-type littermates, in order to identify potential therapeutic targets for schizophrenia treatment. We find that excitatory pyramidal cells in the CA1 region of hippocampus become hyperexcitable in adult Grin2a+/- mice, a change associated with increased input resistance and decreased dendritic complexity and length. These changes to intrinsic cellular properties are accompanied by alterations to glutamate receptor functionality at both entorhinal and CA3 inputs. Behavioural timescale synaptic plasticity is a form of plasticity thought to underlie flexible representations of space in the hippocampal network. Dendritic plateau potentials, which arise from the integration of coincident entorhinal and CA3 input to CA1 pyramidal neurons, are central to BTSP. Therefore, we utilised 2-photon imaging of dendritic calcium events to assess how changes to the schizophrenia risk gene, Grin2a, altered dendritic calcium and synaptic integration in a brain slice preparation. Ultimately, we assessed how a behaviourally relevant form of associative long-term plasticity in CA1 pyramidal cells was affected by heterozygous knockout of the Grin2a gene.

Disclosures: M.D.B. Claydon: None. **M.H. Sabec:** None. **L. alberio:** None. **S. Bajaba:** None. **M.C. Ashby:** None. **D.M. Kullmann:** None. **E.S. Robinson:** None. **J.R. Mellor:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.03/B17

Topic: A.10. Schizophrenia

Support: Stanley Family Foundation

Title: Nr3c2 Loss-of-Function Drives Aberrant Wakefulness, Underpinning Cognitive and Sensory Deficits with Implications for Schizophrenia

Authors: *R. LIN[1], L. THAMARAI KANNAN[2], A. ASAN[2], T. ZHU[2], A. MADDIRALA[2], N. GOBLE[2], N. W. HODGSON[3], J. Q. PAN[2];
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[3]Neurobio., Boston Children's Hosp., Boston, MA

Abstract: Schizophrenia (SCZ) is a severe psychiatric disorder characterized by debilitating positive, negative, and cognitive symptoms, including profound deficits in attention and spatial memory. While dysfunction of thalamocortical circuits and altered brain rhythms are well-established hallmarks, the precise molecular and circuit mechanisms underlying these deficits, particularly during the active "wake" state, remain elusive. We propose a novel concept of "aberrant wakefulness," where the brain fails to establish a coherent, integrated active state, thereby driving core SCZ symptoms.Using an Nr3c2 loss-of-function (LoF) mouse model, we present multi-modal evidence supporting this hypothesis. Bulk RNA-seq revealed a drastic "obscured wakefulness" gene signature in the thalamus during the active period, suggesting a fundamental molecular dysregulation of state. Whole-brain c-Fos mapping uncovered region-specific activity imbalances and altered inter-regional functional correlations. These disruptions were accompanied by behavioral correlates of dysregulated arousal and stress, including increased locomotion and elevated corticosterone levels. Crucially, these multifaceted brain state and circuit alterations manifested as classic neurophysiological deficits in early auditory processing and behavioral deficits in sensory gating, along with profound spatial memory deficits demonstrated by Y-maze and Morris Water Maze impairments.In conclusion, our findings suggest that Nr3c2 dysregulation leads to a state of aberrant wakefulness characterized by multi-level brain dysfunction that directly underlies both sensory processing and severe cognitive deficits. This model provides a novel, integrative framework for understanding SCZ pathophysiology and offers specific molecular and circuit targets for therapeutic intervention.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR057.04/B18

Topic: A.10. Schizophrenia

Support: Medical Research Scotland PHD-50415-2021

Title: Altered cortical synaptome in patients with schizophrenia

Authors: *W. QUAH[1], J. GRIFFITHS[1], B. NOTMAN[1], Z. QIU[1,2], D. DOMINIC[1], K. YUAN[1], C. SMITH[1], S. G. N. GRANT[1];
[1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Sch. of Sci. and Civil Engin., Univ. of Dundee, Dundee, United Kingdom

Abstract: Background: Genetic studies implicate abnormal expression of synaptic proteins as a cause of schizophrenia. The development of synaptome mapping technology in mice, which allows the protein composition, lifetime and nanoarchitecture to be measured reveals immense diversity of synapses, which are spatially distributed at the level of individual neurons, brain regions and the whole brain. The term synaptome describes the diversity of synapse types and subtypes in the brain and the synaptome architecture refers to the spatial distribution of these synapses. The discovery of synaptome architecture raises the hypothesis that the synaptome of the brain is disturbed in schizophrenia. Aim: This study aims to determine how the synaptome changes in patients with schizophrenia. Methodology: This study used postmortem human brain tissue from 8 control samples and 5 schizophrenia samples from the Edinburgh Brain Bank. Four regions were sampled: BA9, BA46, BA41/42, BA19. These regions were chosen as they are implicated in schizophrenia from brain imaging studies. The samples were immunostained with 12 synaptic proteins (PSD95, PSD93, GLUN1, synaptophysin, VGAT, SV2A, gephyrin, DRD2, CACNA1C, GLUA2, GLUN2A, VGLUT1). These markers are a mix of gold-standard synaptic markers, and also schizophrenia risk markers (e.g., CACNA1C, SV2A). We surveyed the synaptome using a mix of pre-, post-, excitatory, and inhibitory synaptic markers. Using fluorescent immunohistochemistry, spinning disc confocal microscopy, and machine learning algorithms, we characterised different synapse types and subtypes in the human brain. We developed synaptome mapping technology for human postmortem brain tissue which allows us to systematically quantify the molecular types and subtypes of excitatory and inhibitory synapses in the neocortex. Results: We observed significant changes in specific pre- and post-synaptic proteins, as well as excitatory and inhibitory proteins in patients with schizophrenia. The effect of schizophrenia varied across regions and synaptic proteins, as some were more vulnerable to changes than others. Layer-specific and region-specific effects on PSD95 and PSD93 were observed in schizophrenia samples as well. Conclusion: Our findings suggest that schizophrenia is a ‘synaptome disorder” arising from the pathology targeting specific synapse types and subtypes. This study is limited by the low number of samples and future studies aim to increase the number of patients examined. Synaptome mapping technology is a powerful tool for identifying synapse pathology.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.05/B19

Topic: A.10. Schizophrenia

Title: The effect of kynurenic acid (KYNA) release on glutamatergic transmission in the hippocampus - A model of schizophrenia

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Abstract: Schizophrenia is a psychiatric disorder that has debilitating symptomatic manifestations, including psychotic episodes, depression-like symptoms, as well as cognitive dysfunction, all three of which contribute to the heavy disease burden for the individual. Multiple hypotheses have been developed in hope of understanding the dysfunctional neurotransmission underlying schizophrenia. Among them is the hippocampal hyperactivity model of schizophrenia, which proposes an imbalance in excitatory and inhibitory transmission in the hippocampus, causing an acute increase in glutamatergic transmission that eventually shift into excitotoxicity and the degeneration of the hippocampus in an early phase of the disease. Kynurenic acid (KYNA) is a neuroactive metabolite of the kynurenic pathway that has been identified as a possible initiator of schizophrenia pathophysiology. Pharmaceutical NMDA receptor- antagonists, such as phencyclidine (PCP), have the ability to induce psychotic and cognitive symptoms similar to the ones seen in schizophrenia patients. As an endogenous NMDA receptor- antagonist, KYNA may have the ability to replicate the effect on glutamatergic transmission that leads to the emergence of above-mentioned symptoms. It was hypothesized that KYNA contributes to the dysregulation in glutamatergic transmission that is observed in schizophrenia pathophysiology, and that the administration of an inhibitor of KYNA synthesis has the ability to possibly reverse pathophysiology. The aim of this study was to investigate the effect of stimulated KYNA synthesis on glutamatergic transmission in the hippocampus of Sprague Dawley rats, using electrophysiological techniques extracellular recording as well as calcium-imaging. The study also aimed to investigate the effect of inhibiting KYNA production on glutamatergic transmission in the hippocampus following KYN stimulation. The results show that stimulating KYNA synthesis in hippocampal slices increases the level of glutamate release in the CA1 region of the hippocampus, and facilitated NMDA-mediated transmission in both the CA1 and CA3 regions of the hippocampus. The inhibition of KYNA production removed the facilitation effect of KYN on NMDA-mediated transmission at certain concentrations. Thus, the kynurenic pathway and its neuroactive metabolites pose an attractive potential target for future pharmaceutical treatment and improved clinical management of schizophrenia.

Disclosures: K.E. Jardemark: None. **S.K. Erhardt:** None. **G.C. Engberg:** None. **K. Lindelöf:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR057.06/B20

Topic: A.10. Schizophrenia

Support: RM1MH133065

Title: Characterizing schizophrenia-associated loss-of-function TRIO mutation in human neurons

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Abstract: Schizophrenia is a severe neuropsychiatric disorder characterized by disruptions in perception and cognition, with substantial heritability and limited effective treatments. Genetic studies have identified TRIO—a guanine nucleotide exchange factor regulating Rho-family GTPases and cytoskeletal dynamics-as a high confidence risk gene for schizophrenia. In mouse models, it has been observed that TRIO haploinsufficiency leads to microcephaly and deficits in neuronal architecture and synaptic transmission. However, the cellular mechanisms by which TRIO mutations contribute to disease pathology remain poorly understood in a human context. Advancements in induced pluripotent stem cells (iPSC) and iPSC-derived neurons are currently the most practical and widely used method for studying the functional effects of specific genetic variants in human neurons. This study investigates the effects of isogeneic heterozygous TRIO loss-of-function (LoF) mutations in human neurons derived from a panel of induced pluripotent stem cell lines from three diverse genetic backgrounds. Induced glutamatergic (EX-iN) and GABAergic neurons (INH-iN) were co-cultured together to closely mimic brain composition in 2D culture system for 5 to 9 weeks. Integrated analysis of neuronal morphology, function, and transcriptomic landscape were performed to characterize neuronal pathologies associated with TRIO LoF mutation. Morphological assessment included immunohistochemistry using trans-synaptic markers and sparse fluorescent labeling to quantify synapse formation, cell structure, and dendritic complexity in EX-iN TRIO LoF found increased synaptic puncta density and altered dendritic complexity. Functional activity was evaluated using high-throughput live-cell imaging of calcium dynamics with the genetically encoded sensor jGCaMP8f. Quantification of calcium activity showed an increase in frequency and amplitude of calcium spikes, indicating aberrant network activity. To map transcriptional changes elicited by TRIO LoF, single-nucleus RNA sequencing was conducted revealing significant transcriptomic enrichment in pathways linked to synaptic transmission and cytoskeletal regulation. Examination of downstream signaling pathways further show altered RhoA and Rac1 protein levels, supporting a shift in cytoskeletal regulation. Targeting Rho-family GTPases pharmacologically, could be a potential target pathway for rescuing TRIO associated neuronal pathology. This study will uncover mechanistic insights into TRIO linked schizophrenia and provide a human-relevant platform for therapeutic discovery for neuropsychiatric disorders.

Disclosures: **P. Gowda:** A. Employment/Salary (full or part-time): Rutgers biomedical health and sciences. **C. Thapa:** None. **S. Diaz de Leon-Guerrero:** None. **H. Zhang:** None. **M. ElAchwah:** None. **A. McCarroll:** None. **R.P. Hart:** None. **J. Duan:** None. **Z.P. Pang:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Topic: A.10. Schizophrenia

Support: National Research, Development and Innovation Office TKP2021-EGA25
National Research, Development and Innovation Office 2017-1.2.1-NKP-2017-00002

Title: Probing the biological consequences of a previously undescribed de novo mutation of ZMYND11 in a schizophrenia patient by CRISPR genome editing and induced pluripotent stem cell based disease-modeling

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Abstract: Background: Schizophrenia (SCZ) is a severe neuropsychiatric disorder of complex, poorly understood etiology, associated with both genetic and environmental factors. De novo mutations (DNMs) represent a new and heavily researched source of genetic variation in SCZ, however, in most cases their biological significance remains unclear. We sought to investigate molecular disease pathways connected to DNMs in SCZ by combining human induced pluripotent stem cell (hiPSC) based disease modeling and CRISPR-based genome editing. **Methods:** Based on exome-sequencing results we selected a SCZ case-parent trio with the case individual carrying a potentially disease causing 1495C>T nonsense DNM in the zinc finger MYND domain-containing protein 11 (ZMYND11), a gene encoding a chromatin-reader and transcriptional corepressor that has also been implicated in biological processes relevant for SCZ. In the patient-derived hiPSC line the mutation was corrected using CRISPR, while monoallelic or biallelic frameshift mutations were introduced into a control hiPSC line. Isogenic cell lines were differentiated into hippocampal neuronal progenitor cells (NPCs) and functionally active dentate gyrus granule cells (DGGCs). Immunofluorescence microscopy and RNA sequencing were used to test for morphological and transcriptomic differences at NPC and DGCC stages. Functionality of neurons was investigated using calcium-imaging and multi-electrode array measurements. **Results:** Morphology in the mutant hippocampal NPCs and neurons was preserved, however, we detected significant transcriptomic and functional alterations. RNA sequencing showed massive upregulation of neuronal differentiation and synaptic machinery genes, and downregulation of cell adhesion genes. Decreased reactivity to glutamate was demonstrated by calcium-imaging. **Conclusions:** Our findings lend support to the

involvement of glutamatergic dysregulation in the pathogenesis of SCZ. This approach represents a powerful model system for precision psychiatry and pharmacological research.

Disclosures: J. Réthelyi: None. **C. Tordai:** None. **A. Apati:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Topic: A.10. Schizophrenia

Support: R01MH111601
NARSAD Independent Investigator Award
5T32MH016804
F32MH120907
K01MH128763

Title: Patterns of Differential Splicing and Associated Putative Effects on Protein-Protein Interactions in the Striatum in Psychosis

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Abstract: Psychosis is a highly disruptive and often debilitating symptom found in disorders such as schizophrenia and bipolar disorder. Prior studies have heavily implicated the striatum in psychosis. Furthermore, previous studies have reported a dysregulation of alternative gene splicing in psychosis. Alternative splicing is a major factor in transcriptional diversity and little research has been done regarding the intersection of psychosis and splicing in the striatum. Here, we begin to elucidate differential splicing (DS) in the striatum. To accomplish this, RNA-seq was performed on nucleus accumbens (NAc), caudate, and putamen samples from subjects with psychosis (n=36) or matched unaffected subjects (n=36). For identification and visualization of local splice variation we used LeafCutter and LeafViz, respectively. DIGGER was used to analyze how domain-domain interactions impact protein-protein interactions and Metascape was used for pathway enrichment.
Across the three regions, we identified 705 named, unique genes that were DS. In the NAc, these genes were related to cellular organization and transport. In the caudate, there was enrichment in immune and cellular organization pathways. In the putamen, cilia-, mitochondria-, and cellular organization-related pathways were DS. Furthermore, there were many genes implicated in psychosis that featured cases of differential splicing NRXN1, NRXN2, and CACNA1C. In these

genes, the corresponding exons involved in the DS events encoded protein domains that participate in protein-protein interactions, potentially leading to alterations in the viability of these interactions. This may cause downstream effects in vital processes, such as bridging the synaptic cleft and the regulation of calcium ion channel activity. Future studies regarding differential transcript usage, splice factors, day/night differences, and sex differences will help us better understand striatal DS and its potential functional consequences in psychosis.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR057.09/B23

Topic: A.10. Schizophrenia

Support: NIH GRT-00004264

Title: Schizophrenia is associated with impaired neuronal mitophagy in 22q11.2 deletion syndrome

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Abstract: The 22q11.2 deletion syndrome (22q11DS) is a major genetic risk factor for schizophrenia (Sz), a debilitating illness that affects roughly 1% of the population worldwide. We previously found, in both human stem cell-derived neurons (iNs) and lymphoblastoid cell lines, that the presence of Sz in 22q11DS is linked to weaker mitochondrial energetics, while the absence of Sz is associated with upregulated expression of mitochondrial (mito) biogenesis and turnover genes, suggesting a compensatory mechanism. Here, we focused our current study on mitophagy; our goal was to determine whether mito biogenesis and turnover is indeed enhanced in the 22q11DS non-psychotic group (-Sz), and why the psychotic group (+Sz) fail to undergo this compensation.We find that mito health, as measured by mitochondria inner membrane potential, is compromised in iNs from 22q11DS+Sz group compared to both the 22q11DS -Sz group and neurotypical controls. Analyzing an in-house modified MitoTimer protein, we detect the presence of a population of aged mitos in 22q11DS +Sz group, suggesting impairment of degradation, while the mito population of the 22q11DS -Sz group is younger than controls, consistently with enhanced mitobiogenesis in this group. RNA-sequencing of iNs identifies a remarkable enrichment of downregulated genes associated with lysosomal storage diseases and mitophagy-related pathways in the psychotic group. Proteomic analysis supports this interpretation, as does western blotting for lysosomal proteins. Direct measurement of autophagy

reveals indeed a deficit in the 22q11DS +Sz. Since compounds aimed to restore lysosomal activity are available, we prove that treating the iNs with acidifying nanoparticles restores lysosome function, substantially correcting the autophagy deficit and improving mitochondrial health in 22q11DS +Sz iNs. Hence, our results indicate that an important component of neuronal mito dysfunction, that differentiates individuals with 22q11DS with versus without psychosis, includes mitophagy and lysosomal dysfunction. We propose that enhancement of mitophagy may be a novel treatment or prevention therapeutic opportunity for Sz in the context of 22q11DS.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Program #/Poster #: PSTR057.10/B24

Topic: A.10. Schizophrenia

Support: NIH R25 grant: 1 R25 NS130966-01

Title: Investigating genetic and epigenetic changes associated with schizophrenia

Authors: *T. ROBERTS[1], S. ORELLANA[2], E. KOVACS[5], B. BARAN[2], A. MCCLEERY[2], K. L. PARKER[3], A. J. WILLIAMS[3], T. WASSINK[3], M. E. GAINE[4];
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Abstract: Schizophrenia, a chronic neurological brain disorder, is characterized by symptoms that can be either positive (e.g. hallucinations), negative (e.g. lack of interest), or a combination of both. Another core feature of the disorder is the disorganization of thought and behavior. It affects a projected 1% of the population, making it a significant public health concern. Unfortunately, this condition and its symptoms are still not fully understood. One potential biological mechanism underlying schizophrenia is epigenetics, a DNA modification that occurs without altering the genomic sequence. DNA methylation is a type of epigenetic modification that has previously been linked to psychiatric disorders. Previously, our lab looked at family trios with an offspring with schizophrenia and were able to identify DNA methylation changes associated with immune response and epigenetic aging. This suggests that studying DNA methylation may help us identify potential associations with symptoms of schizophrenia (e.g. sleep disruptions) and begin taking steps at understanding the molecular mechanisms driving schizophrenia and associated symptoms. In this study, we hypothesized that DNA methylation changes throughout the genome are associated with schizophrenia and endophenotypes such as sleep and cognitive deficits. To test this hypothesis, whole blood samples were collected from individuals with schizophrenia (N=24) and non-psychiatric controls (N=24). Samples were age

and sex matched. After extracting DNA, genome-wide DNA methylation profiles were generated using the Illumina EPIC array-based method at the Iowa Institute of Human Genetics, Genomics Division. Analysis was carried out using R studio and the Chip Analysis Methylation Pipeline (ChAMP), allowing for a direct comparison between individuals with schizophrenia and controls. The top finding was hypomethylation in the COX5B gene (uncorrected p-value = 6.45E-07). This gene is the terminal enzyme of the mitochondrial respiratory chain and is also related to inflammation. Schizophrenia has previously been associated with pro-inflammatory substances in the blood and brain and this work also supports our previous study in trios. Future work will include expanding our sample size as blood collection is ongoing. The long-term goal of this work is to identify dysregulated genes and biological pathways associated with schizophrenia that may be novel therapeutic targets.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Program #/Poster #: PSTR057.11/B25

Topic: A.10. Schizophrenia

Support: CIHR PJT 190246

Title: Developmental deficits in ventral hippocampal parvalbumin interneurons induce schizophrenia phenotypes in mice

Authors: *E. J. MAES[1], S. K. BHARDWAJ[3], M. NATH[3], T. WONG[4], M. P. BRANDON[2], L. K. SRIVASTAVA[5];
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Abstract: Structural and functional abnormalities in the anterior hippocampus, thought to be of developmental origin, are consistently reported in individuals with schizophrenia. Specifically, postmortem patient tissue shows a significant reduction in parvalbumin (PV) interneurons in the hippocampus. Rodent studies have shown that the ventral hippocampus (VH; analogous to primate anterior hippocampus) strongly influences cognitive, social and emotional behaviors impaired in schizophrenia. We hypothesized that juvenile ablation of PV neurons in VH would result in adult behavioral and neural phenotypes associated with schizophrenia. We investigated the behavioral and neural consequences of juvenile diphtheria toxin A (DTA)-induced ablation of PV neurons. AAV2/8.EF1a.mCherry.flex.DTA (AAV-DTA), or control virus AAV2/8.EF1a.flex.mCherry were injected bilaterally in the VH of postnatal day 14 PV-Cre

mice. Amphetamine-induced locomotor activity, social interaction, anxiety-like behavior, spatial memory, and novelty/habituation were assessed in males and females at young adulthood. Whole-cell recordings in the mPFC and nucleus accumbens dopamine sensor fiber photometry recordings were performed. Developmental ablation was also compared to acute inhibition using hM4Di DREADDs to determine the role of PV neurons in long term circuit adaptation. DTA ablation resulted in a progressive loss of PV-expressing neurons in VH and resulted in increased cFos-labeled cells, indicating hyperexcitability of the VH. Additionally, ablation enhanced d-amphetamine-induced locomotor activity, indicating hyperdopaminergic state. Behavioral tests displayed impairments in reversal learning and memory, as well as Y-Maze performance. PV ablation also induced deficits in social novelty memory and anxiety-like behaviors. Furthermore, ablated animals had impaired latent inhibition, that is, they learned about habituated cues as if novel. Acute DREADDs inhibition of VH PV neurons was sufficient to impair latent inhibition and induce persistent phasic dopamine release to familiar cues. Our data suggest that juvenile disruption of VH PV is sufficient to induce cognitive-behavioral and neural phenotypes of schizophrenia in adulthood.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Program #/Poster #: PSTR057.12/B26

Topic: A.10. Schizophrenia

Support: NIMH T32MH01533

Title: Endophenotypes of Schizophrenia Based on Treatment Response in Task-Related and Resting-State Electroencephalography

Authors: *A. BODNAR[1], D. HAUKE[3], J. RODRIGUEZ-SANCHEZ[4], L. HUESTEGGE[3], C. A. ROSS[5], E. J. GOLOB[6], R. L. MARGOLIS[2], R. A. ADAMS[3], F. C. NUCIFORA[1];
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Abstract: Schizophrenia is a heterogeneous disorder. While ~70% respond to standard antipsychotics (nonTRS), ~30% are treatment-resistant (TRS) and typically require clozapine. Up to 30% of TRS patients fail to respond to clozapine, forming the ultra-treatment-resistant (UTRS) group. We hypothesize that nonTRS, TRS, and UTRS represent distinct neurobiological endophenotypes detectable via EEG.We applied three paradigms—visual Stroop, passive auditory oddball, and a novel auditory spatial Stroop—to assess ERP (P200, P300), spectral power, and dynamic causal modeling (DCM) across nonTRS (N=10), TRS (N=14), UTRS

(N=13), and healthy controls (HC, N=10). PANSS scores: nonTRS=41.4, TRS=48.4, UTRS=83.8, HC=30.5. Resting-state EEG (eyes-open/closed) was also analyzed.Our preliminary results show TRS patients with enhanced P300 during visual Stroop ($p<0.001$) and increased P200 to deviant tones in auditory oddball ($p=0.006$). TRS also exhibited stronger intrinsic and extrinsic fronto-temporal DCM connectivity. In contrast, UTRS showed elevated P300 during auditory spatial Stroop ($p=0.003$), reduced DCM connectivity driven by cortical inhibition ($p=0.007$), and increased resting-state low gamma power (31-40Hz; $p<0.05$). TRS and UTRS both showed elevated delta/theta power ($p<0.05$). A robust right-ear advantage in reaction time ($p<0.0001$) was observed across groups, consistent with hemispheric language asymmetries.These preliminary findings reveal potentially distinct neurophysiological signatures in schizophrenia subtypes. One interpretation is that enhanced ERP responses and fronto-temporal coupling in TRS is distinct from heightened inhibition and gamma power in UTRS. Analysis of resting-state EEG may provide additional information on the distinctions among nonTRS, TRS, and UTRS subtypes of schizophrenia. Overall, EEG-based biomarkers may support precision psychiatry by identifying subtype-specific neurobiological pathophysiology and informing treatment strategies for individuals with schizophrenia.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

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Topic: A.10. Schizophrenia

Title: Molecular Profiling of Cerebellar Vermis Tripartite Synapses in Psychiatric Disorders

Authors: *M. E. CORTES-GUTIERREZ[1], S. GANDSAS[4], A. CHANDIA CRISTI[2], X. E. FAN[3], N. AYITHAN[3], M. TRAINOR[3], R. O'HARA-PAYNE[5], R. JOHNSON[6], R. SCHWARCZ[7], S. A. AMENT[8];
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Abstract: *The human cerebellum is a region of the brain that processes information from a variety ofsensory inputs. These signals are integrated to generate responses that involve memory,sensory perception, and motor coordination. A key component in this informationprocessing is the synapse, a specialized junction through which neurons communicate.Synapses are composed of thousands of proteins that function as receptors, ion*

channels,and transporters. For an appropriate response to a stimulus, synaptic communicationmust be well-established, adaptable, and tightly regulated. In the presenceof mental illnesses such as schizophrenia (SCZ) or bipolar disorder (BP), the molecularcomposition and function of synapses can be significantly altered.This study utilizes human postmortem tissue from the cerebellar vermis, a regionimplicated in emotional processing. We focus on the tripartite synapse, wherea parallel fiber synapses onto the dendrite of a Purkinje cell, andthe Bergmann glia ensheathes the synaptic structure. Through transcriptomic analysis, weidentified significant differences in Robo2 transcripts in Bergmann glia. Robo2 is part of thesynaptic architecture but whose specific role in synaptic function remains poorlyunderstood.In our approach, using enriched synaptosomes fractions from specific tripartite synapses,we assess at the protein-level, the differences across healthy, SCZ, and BP subjects wefound in their transcriptome. Furthermore, we compare these glia-associated synapses tothose lacking glial components, aiming to uncover the specific contributions of glial cellsto molecular alterations in the synapse that may be linked to psychiatric disorders

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Program #/Poster #: PSTR057.14/B28

Topic: A.10. Schizophrenia

Title: The impact of the schizophrenia-associated 3q29 deletion on human cerebellar development

Authors: *R. SEBASTIAN, M. SCARNATI, J. MULLE;
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Abstract: The 3q29 deletion syndrome (3q29del) is a rare, high-risk copy number variant (CNV) caused by a 1.6 Mb deletion encompassing 21 genes. This deletion confers a 40-fold increased risk for schizophrenia and also elevates risk for autism spectrum disorder (ASD), intellectual disability, ADHD, and anxiety disorders, making it a high-value target for investigating molecular and cellular mechanisms that underlie neurodevelopmental disorders. In our recent study, we performed neuroimaging on human study participants with 3q29del, revealing a decrease in cerebellar volume. Moreover, preliminary findings from our group indicate that transgenic 3q29del mice also have a smaller cerebellum, suggesting this is a cross-species impact of the deletion. We hypothesize that mechanisms governing normal cerebellar development are altered by the 3q29 deletion. Given that the cerebellum is a pivotal structure in driving motor tasks and cognitive behaviors, there is a crucial need for a robust model to investigate the mechanisms by which 3q29del modulates human cerebellar development. Recent advancements

in stem cell technology have enabled the use of cerebellar organoids, a powerful *in vitro* 3D model for interrogating human cerebellar development. In our study, we have employed a human stem cell-derived cerebellar organoid protocol to measure cerebellar growth and downstream molecular changes. We first ensured the reproducibility of the protocol in our hands, assessing for canonical cerebellar markers over the development of the organoids using control iPSC lines. Next, we have grown cerebellar organoids from 2 3q29del patient-derived iPSC lines alongside 2 sex -matched control lines to measure to trajectory of cerebellar growth. Our preliminary comparison from male subjects shows smaller growth in the 3q29del line as compared to control. However, an opposite effect is observed for the cerebellar organoids grown from a female individual with the deletion: the 3q29del cerebellar organoid is larger than the control. These data suggest there may be sex-dependent effects that modulate early cerebellar development in the 3q29del background. Continuing efforts in our study include investigation of the molecular changes that underlie sex differences in cerebellar growth in the 3q29del background, including RNA and protein expression over the development of the cerebellar organoids. These preliminary data are foundational to understanding the pathophysiology of 3q29 deletion syndrome, and advance the development of more precise, individualized treatment strategies for affected individuals.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR057.15/B29

Topic: A.10. Schizophrenia

Title: Altered Microglial Morphology and Functioning in a 3q29 Deletion Mouse Model of Schizophrenia

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Abstract: Schizophrenia (SCZ) is a mental disorder characterized by disruptions in thought processes, perception, emotional responsiveness, and social interactions. While the severity of symptoms varies among individuals, SCZ can be debilitating. One major category of SCZ risk factors involves genetic alterations. We used 3q29del mice, a high-risk genetic model for SCZ with a ~40-fold increased likelihood of developing the disease. Both genders were for our research and as controls we used the littermates without deletion. Microglia are the main immune cells of the brain. It is evident that microglia play a role in SCZ development; however, the nature of that role remains unclear. Our research aims to help clarify the involvement of microglia in SCZ. First, we characterized microglial morphology at three time points (P7, P28, and P63) in different areas of the cortex using immunohistochemistry with Iba1 to investigate

potential developmental differences. (N = 6 of 3q29del; N = 10 control per each timepoint) The parameters we evaluated included Cell Territory (a polygon created by connecting the outermost points of the cell), Cell Volume, Ramification Index (to assess cellular complexity), and Average Primary Branch Length. Our results show that postnatal microglial maturation is altered in 3q29del mice. While differences were already observable at P7, they became much more pronounced by P63, with microglial cells being smaller, less complex, and covering less brain volume. Additionally, we performed co-staining with Iba1 + DAPI + CD68 + P2Y12 to assess activation/resting features. Morphologically, microglia do not appear activated; however, they express high levels of CD68, a marker of phagocytic activity. We also used Iba1 + DAPI + TMEM119 + CD206 to exclude macrophage infiltration. At present, we believe macrophage infiltration is insignificant compared to that in control animals. Next, we assessed microglial physiology using in vivo two-photon imaging in layer 1 of the primary somatosensory cortex barrel field. Normal microglial function depends heavily on the movement of its processes. While the movement of process tips did not appear as impaired as expected, the overall movement patterns and speed distribution were altered. In conclusion, our results demonstrate substantial morphological alterations developing postnatally, corresponding with the typical onset of SCZ in early adulthood. These microglial abnormalities could affect circuit formation and function, potentially contributing to SCZ symptoms.

Disclosures: N. Rozhkova: None. **N. Fukatsu:** None. **H. Wake:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.16/B30

Topic: A.10. Schizophrenia

Support: NRF-2021R1C1C1012901
RS-2025-00554040

Title: Regional cortical thinning in multiple-episode psychosis compared to first-episode psychosis and its association with symptom severity

Authors: *M. BANG[1], B. KIM[2], N. KANG[3], S.-H. LEE[1];
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Abstract: Background: Schizophrenia is associated with structural brain abnormalities, including reduced cortical thickness, which reflects underlying cytoarchitectural changes. Recurrent psychotic episodes may contribute to progressive cortical thinning, potentially leading to more severe clinical symptoms. This study aimed to compare cortical thickness between first-episode psychosis (FEP) and multiple-episode psychosis (MEP) patients and to examine

associations with symptom severity.**Methods:** One hundred twenty-five FEP (89 women) and 81 MEP participants (53 women) underwent T1-weighted MRI scans. Cortical thickness was measured using FreeSurfer. Clinical symptoms were assessed using the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS). Group comparisons of cortical thickness were conducted with age, sex, and intracranial volume as covariates.**Results:** Compared to FEP participants, MEP participants showed significantly reduced cortical thickness in the right superior frontal gyrus ($p < 0.004$) and left precuneus ($p < 0.001$). Thinner right superior frontal cortex was significantly associated with higher SAPS delusion scores ($p = 0.015$) and SANS affective blunting scores ($p = 0.041$). Thinner left precuneus was also correlated with higher affective blunting scores ($p = 0.032$).**Conclusion:** MEP participants exhibited reduced cortical thinning in key frontal and parietal regions compared to FEP participants. These structural alterations were associated with more severe positive and negative symptoms. The findings support the hypothesis that recurrent psychotic episodes contribute to progressive brain changes, which may underlie worsening clinical outcomes. Early intervention to prevent relapse may be critical for minimizing neuroanatomical deterioration in individuals with psychosis.

Disclosures: M. Bang: None. **B. Kim:** None. **N. Kang:** None. **S. Lee:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.17/B31

Topic: A.10. Schizophrenia

Support: Western University of Health Sciences

Title: Genomic analysis of a schizophrenia cohort shows deleterious variants mainly affect neuropathology-related genes rather than schizophrenia-linked genes

Authors: *A. BASKYS;
Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Rationale. Research on the impact of schizophrenia-associated genes on clinical presentation, particularly treatment-resistant negative symptoms, is limited. This study used Whole Exome Sequencing data from the Sweden-Schizophrenia Population-Based Case-Control cohort (phs000473.v2.p2, male, 18-62 years old, all data are from dbGaP, controlled access protocol No. 24624) to examine schizophrenia-related genes. Methods. FASTQ files were annotated using SnpEff and SnpSift, and variants were called with FreeBayes. rsIDs were retrieved from dbSNP, allele frequencies from GnomAD, variant pathogenicity from ClinVar, and predictor values from dbNSFP. Variants classified as Benign/Likely Benign or with allele frequency >5% were excluded. Variants were designated as "deleterious" based on allele frequency, predictors, conservation, and clinical significance using an in-house algorithm. Genes

carrying deleterious variants were mapped to organ systems using DisGeNET and UMLS Metathesaurus, ranked by gene-disease association scores from DisGeNET, and associated pathways analyzed using g:profiler, a functional enrichment tool. Results. To validate our algorithm, we compared the number of cases with epilepsy-related genes carrying deleterious mutations in an epilepsy cohort ("neurological phenotype", N=193, phs001489.v3.p2) and a Crohn's cohort ("non-neurological phenotype", N=111, phs001076.v1.p1). We found 173 such cases (89.6%) in the epilepsy cohort and 13 cases (11.7%) in the Crohn's cohort. In the schizophrenia cohort, only 27/142 cases (19.0%) had deleterious variants in schizophrenia-associated genes, while 122/142 cases (85.9%) carried deleterious variants in genes linked to nervous system diseases associated with brain neuropathology. Enrichment analysis showed schizophrenia-associated genes were linked to glutamatergic or dopaminergic synaptic transmission. Nervous system diseases-associated genes known to be implicated in cortical neuronal loss in frontal and parietal lobes, mapped to Human Phenotype Ontology terms "dementia", "cognitive impairment", "diminished motivation" and biological processes such as "mitochondrial organization" or "cellular respiration". Conclusions. These findings indicate that a majority of schizophrenia patients may have deleterious variants in genes linked to neuropathological processes rather than synaptic dopaminergic pathways. They suggest, that future development of effective pharmaceuticals for treatment of positive and negative symptoms of schizophrenia should rely not only on clinical phenotypes but also on genomic profiles of clinical trial participants.

Disclosures: A. Baskys: None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.18/B32

Topic: A.10. Schizophrenia

Support: Crafoord Foundation
Forska Utan Djurförsök
Åhlen-stiftelsen

Title: CRISPR-based activation of schizophrenia-associated endogenous retrovirus alters human synapses

Authors: *C. DAVIS-HANSSON[1], R. GARZA[1], J. JOHANSSON[1], L. CASTILLA-VALLMANYA[1], V. BUSSKAMP[2], J. JAKOBSSON[1], E. JOHANSSON[1];
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Abstract: Human endogenous retroviruses (HERVs) are ancient remnants of retroviral infections that have expanded in our genome through retrotransposition. HERVs make up 8% of

the human genome and some HERV proviral fragments contain the viral genes *env, pro, pol,* and *gag*. While the majority of HERVs are no longer expressed, there are examples of viral particles from HERVs that have been co-opted including the envelope protein syncytin-1 which mediates cell to cell fusion in the placenta of primates. Dysregulation of HERVs has been associated with neurological and psychiatric disorders including schizophrenia. A study of note found envelope proteins from the HERV-W subfamily in patients with schizophrenia and bipolar disorder; expression of that protein in rats disrupts the maturation of glutamatergic synapses and induces behavioral abnormalities attributed to psychosis. HERV-W, however, is primate-specific, so it is unclear whether expression of the envelope protein would have the same effect in human glutamatergic neurons as it does in rats. To determine the effects of HERV-W expression in human glutamatergic neurons, we utilized a two-plane co-culture system of human iPSC-derived glutamatergic neurons (iNGNs) and CRISPR- activation to directly target the HERVW family. We generated iPSC cell lines with a doxycycline-inducible Neurogenin-1 and Neurogenenin-2 gene cassette and co-cultured with astrocytes in separate planes. We found that the iNGNs are an appropriate model as they express the neuronal marker MAP2 by 6 days post-induction (dpi) and express the glutamatergic marker vGLUT. The cells exhibit spontaneous action potentials by 30 dpi, and show progressive increases in post-synaptic density up to 72 dpi. For CRISPR activation, we constructed a dCas9-VP64 CRISPRa system and designed guide RNAs targeting the promoter regions of the HERV-W family which allows activation of the elements in their endogenous regulatory landscape. Preliminary results show that, at 30 dpi, HERV-W activation resulted in altered gene expression related to glutamatergic synapses (e.g., **CAMK2A, DLG4, SYNGAP1**) and risk genes for schizophrenia (e.g., **CACNA1C, ATP1A3, SHANK3**). Furthermore, the transcriptional alterations were accompanied by a significant reduction in synaptic density. Together, these preliminary findings demonstrate that HERV-W activation in human glutamatergic neurons is sufficient to induce alterations in excitatory synapses. We are conducting additional experiments to further explore how HERV-W activation in human glutamatergic neurons may contribute to the pathophysiology of schizophrenia in some patients.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.19/B33

Topic: A.10. Schizophrenia

Support: R01MH122556
1R21DE032806
1RM1MH138309

Title: Bridging Brain Development and Vasculature: Investigating Shared Molecular Pathways in Neuropsychiatric Disorders

Authors: *T. PURI[1], Y. MAO[2];
[2]Biol., [1]Penn State Univ., University Park, PA

Abstract: Neuropsychiatric disorders such as schizophrenia are increasingly recognized to involve complex interactions between brain developmental processes and vascular function. Disruptions in neurodevelopment, synaptic signaling, and endothelial integrity may converge to influence disease risk and progression. This study employs an integrative systems biology approach to analyze publicly available molecular datasets across brain and vascular tissues. By examining gene networks, co-expression patterns, and genetic associations, the work aims to identify common biological pathways that link neurodevelopmental mechanisms with vascular dysfunction. Understanding these cross-tissue molecular connections offers insight into the interplay between neural and vascular systems and provides a foundation for exploring novel targets relevant to neuropsychiatric disease etiology.

Disclosures: T. Puri: None. **Y. Mao:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.20/B34

Topic: A.10. Schizophrenia

Support: T32GM148383
MSCRF
BBRF
Lieber Institute

Title: Setd1a Regulates Psychiatric Gene Networks Involved In Synaptic Function and Genome Stability in Rare and Common Forms of Schizophrenia

Authors: *J. A. ERWIN[1], T. SAWADA[2], D. R. WEINBERGER[2], A. C. PAQUOLA[3], H. GILES[4];
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Abstract: Rare loss-of-function (LoF) mutations in SETD1A are associated with schizophrenia (SCZ). However, how SETD1A haploinsufficiency leads to SCZ-associated phenotypes and its relevance to patients without these rare mutations is unknown. Here, we identify SETD1A bound loci and regulated genes in human prenatal cortex and isogenic pluripotent stem cell-derived neuronal models engineered with SETD1A LoF variants, including the most common patient mutation. SETD1A preferentially binds the promoters of polygenic risk loci for psychiatric disorders that regulate chromatin remodeling, DNA repair, and synaptic function. Additionally, SETD1A binds to DNA damage prone sites in neural progenitor cells and postmitotic neurons.

SETD1A haploinsufficiency causes accelerated neurogenesis, reduced neuronal complexity, and DNA damage accumulation in postmitotic neurons that is rescued by inhibiting the H3K4me2/3 demethylase KDM5. In postmortem SCZ cortical tissue, individuals who lack SETD1A mutations exhibit reduced SETD1A expression, associated with downregulation of SETD1A-regulated genes, implicating SETD1A-H3K4me dysfunction in sporadic SCZ cases. We demonstrate that SETD1A functions as a convergent upstream modulator of a gene network that underlies polygenic risk for psychiatric disorders, which suggests that restoring the SETD1A-H3K4 methylation epigenetic imbalance may benefit a broad psychiatric population.

Disclosures: J.A. Erwin: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aluco Biosciences. F. Consulting Fees (e.g., advisory boards); Aluco Biosciences. **T. Sawada:** None. **D.R. Weinberger:** None. **A.C. Paquola:** None. **H. Giles:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.21/B35

Topic: A.10. Schizophrenia

Title: Alterations in tryptophan-kynurenine metabolism and its correlation with subclinical inflammation in living and post-mortem cohorts of schizophrenia patients from the indian population

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Abstract: Background: Schizophrenia (SCZ) manifests with diverse symptoms, reflecting its complex pathophysiology. While classical neurotransmitters are well studied, the role of kynurenine pathway (KP) metabolites arising from tryptophan catabolism is less understood. Dysregulation of KP impacts metabolite levels, contributing to neuroinflammation, neurodegeneration, and cognitive dysfunction. This study investigates KP metabolites and enzyme expression (mRNA and protein), along with cytokine profiles at tissue and systemic levels in an Indian SCZ cohort to identify potential biomarkers and therapeutic targets. **Methods:** Post-mortem (PM) dorsolateral prefrontal cortex (DLPFC) samples from 10 SCZ patients and 17 healthy controls (HCs) were obtained from the Human Brain Tissue Repository. Serum samples from living SCZ patients both drug-naïve (DN) and risperidone-treated (RT)—and HCs were collected at NIMHANS, Bangalore. KP metabolites were measured by LC-MS/MS; cytokines by ELISA. PM tissue was analyzed for KP metabolites, gene expression via RT-qPCR, and protein levels of key enzymes via western blotting. **Results:** In both the living and PM cohorts, we

observed significant differences in peripheral and tissue KP metabolite levels ($p < 0.001$ and $p < 0.05$ for DN and RT SCZ). The peripheral cytokine levels of the DN and RT SCZ groups differed significantly from those of the controls ($p < 0.001$). At both the mRNA and protein levels, SCZ patients had significantly higher tissue expression of the TDO gene, with a 2.5-fold increase compared to healthy controls ($p < 0.001$). Additionally, there was an increase in KAT II gene expression in the SCZ group ($p < 0.05$). SCZ patients had greater mRNA levels of the pro-inflammatory cytokines TNF-α and IL-1β ($p < 0.001$) than HC patients. Furthermore, protein levels of KAT II and IDO ($p < 0.05$) were significantly increased in the SCZ cohort relative to the control group. **Conclusion:** Our findings suggest that KP dysregulation and low-grade inflammation contribute to SCZ pathology. Elevated TDO, KAT II, IDO, TNF-α, and IL-1β support a neuroimmune basis for symptomatology. This work highlights the utility of KP and cytokine profiles as biomarkers and therapeutic targets, especially within the genetically diverse and metabolically vulnerable Indian population.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

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Topic: A.10. Schizophrenia

Support: A Grant-in-Aid for Scientific Research (S) to T. Isa (22H04992)
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Title: Disc1-deficient macaque model: a novel platform for dissecting the neurobiological basis of psychiatric pathophysiology via multidimensional analyses

Authors: *Z. LAN[1,4,2], T. TSUKIYAMA[4,5], K. HIKISHIMA[6], N. OISHI[3], C.-Y. CHEN[7,4], Y. SHINTO[4], S.-I. URAYAMA[3], K. ISHIZUKA[8], A. SAWA[8,2], H. ONOE[3,9], T. ISA[1,4,3];
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Abstract: Psychiatric disorders have complex etiologies involving genetic and environmental factors, making them arduous to model in animals such as rodents, particularly due to the

diagnostic limitations and the species-specific aspects of neuroanatomy. *Disrupted-in-schizophrenia 1* (*DISC1*), a key regulator of neurodevelopment, functions as a hub-molecule in the brain and is crucial for understanding the biological essence of psychiatric pathophysiology. To investigate the impact of DISC1 dysfunction, we employed a genetically engineered macaque model (*Macaca fascicularies*) with disrupted DISC1 and conducted an integrated analysis combining behavioral assessment via the Human Intruder Test (HIT) with resting-state fMRI (rsfMRI) to examine brain functional connectivity. In the HIT, DISC1 KO macaques (n = 5) exhibited markedly reduced external responses, such as anxiety and excitation, compared to wild-type controls (WT, n = 4). Conversely, they displayed prolonged internally-triggered behaviors including exploratory actions, suggesting impaired reactivity to social stimuli and a preference for self-directed engagement. RsfMRI revealed pronounced disruptions in brain network organization in DISC1 KO subjects (n = 5), characterized by reduced global functional connectivity strength and density. The deficits were particularly evident in attenuated intra-regional connectivity within the frontal cortices and diminished inter-regional connectivity between the frontal lobes and parietal or temporal areas. Compared to WT (n = 6), the network topology in DISC1 KO animals was more fragmented and sparser with a steeper decline in connectivity. Small-world network analysis indicated reduced local modularity in the DISC1 KO group, while eigenvector centrality analysis showing a shift in network hub configuration, with reduced centrality in the dorsolateral prefrontal cortex and increased dominance of sensory-related regions such as the somatosensory and occipital cortices. This reorganization suggests a top-down processing bias at the expense of prefrontal-mediated regulatory mechanisms, which may reflect behavioral impairments in HIT. This genetically modified macaque model successfully replicated key neuropsychiatric endophenotypes, such as altered socio-emotional features and disrupted functional networks in specific brain regions, which are difficult to capture in rodent models due to the differences in behavior complexity and neuroanatomy compared to humans. As such, this model offers a valuable platform for elucidating DISC1-related pathophysiology and for guiding the development of targeted therapeutic interventions.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.23/B37

Topic: A.10. Schizophrenia

Title: Depolarization affects the bioenergetic state of hiPSC-derived neural progenitor cells (i.e., NPCs)

Authors: *P. DUJMIC[1], P. K. REARDON[2], R. KARMACHARYA[3];
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Abstract: Coupling of neural activity to cellular respiration is essential for neuronal structure and function. Disruptions to activity-regulated metabolism have been implicated in several neurodevelopmental disorders, yet when (and how) neural activity is linked to metabolism during development remains poorly understood. In this study, we explored the impact of mild membrane depolarization on the metabolic state of neural progenitor cells derived from human induced pluripotent stem cells (i.e., hiPSCs). Prior studies implicate electrical stimulation in promoting progenitor maturation, a process involving transition from aerobic glycolysis to oxidative phosphorylation. As such, we anticipated that KCl stimulation would be associated with heightened mitochondrial respiration. Neural progenitor cells (i.e., NPCs) were differentiated from three control and three schizophrenia patient-derived hiPSC lines using dual SMAD inhibition. To induce membrane depolarization, NPCs were exposed to high extracellular potassium levels (i.e., 25 mM) followed by metabolic analysis using the Seahorse Mito Stress test. Paradoxically, we observed a dose-dependent decrease in oxygen consumption with membrane depolarization, suggesting an enhancement of aerobic glycolysis that may instead promote NPC self-renewal. Schizophrenia patient-derived NPCs demonstrated a similar trend with KCl stimulation, albeit slightly lower baseline respiration in comparison to controls. Interestingly, treatment with the antipsychotic chlorpromazine (10 uM) prevented reductions in mitochondrial respiration while attenuating membrane depolarization in patient-derived NPCs. Altogether, these preliminary data suggest a new role for graded potentials in regulating the bioenergetic state of NPCs. A mechanistic understanding of this coupling may advance our understanding of developmental disruptions in psychiatric disease.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.24/B38

Topic: A.10. Schizophrenia

Title: Single-nucleus RNA-seq investigation of schizophrenia reveals neurodevelopmental and synaptic dysfunction in upper and deep layer excitatory neurons of the dlPFC

Authors: *L. SCHRAMM;
Human Brain Collection Core, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Schizophrenia (SCZ) is a psychiatric disorder characterized by hallucinations, delusions, disorganized thought, social withdrawal and dulled affect. It causes long-term disability and early death due to high rates of suicide. Treatments have targeted symptomatology, as the underlying mechanisms of disease are poorly understood. Prior studies have implicated

cell-type specific gene expression changes in excitatory neurons of the prefrontal cortex in the pathogenesis of SCZ, and functional enrichment analyses have implicated neurodevelopmental and synaptic processes. To further elucidate the genomic mechanisms underlying SCZ, we performed single nucleus RNA sequencing (snRNA-seq) using 10X Genomics Chromium Single Cell 3' Platform on the dorsolateral prefrontal cortex (dlPFC) of 23 cases with SCZ (7F, mean age = 55.1+12.3) and 21 controls (7F, mean age = 50.8+14.5). We initially captured 637,000 nuclei, and following quality control measures for doublets, mitochondrial percentage, transcript count and gene count per cell, our final dataset consisted of 393,000 nuclei across 16 excitatory, 19 inhibitory, and 11 non-neuronal cell classes. We conducted differential gene expression analysis controlling for sex, race, post-mortem interval, RNA integrity number, mitochondrial RNA percentage, ribosomal RNA percentage, and cellular detection rate. This analysis revealed transcriptional dysregulation in >3,900 genes (padj < 0.05; |logFC| > 0.228) across all 46 cell types, primarily in superficial (n=1,556, 3 cell types) and deep layer (n=567, 3 cell types) excitatory neurons. Inhibitory neuron subclasses showed minimal differential expression, apart from one class of parvalbumin expressing interneurons (n=233). Gene ontology analysis identified upregulation of genes involved in cell differentiation and downregulation of genes involved in synapse and axon assembly, neurogenesis, and CNS development in superficial and deep layer excitatory neurons. These results suggest involvement of these cell types in dysregulation of cell differentiation and maturation in SCZ. Our ongoing analysis is focused on testing the hypothesis that excitatory neurons undergo divergent or stunted developmental trajectories in SCZ. We conclude that developmental pathways appear dysregulated in superficial and deep layer excitatory neurons in individuals with SCZ.

Disclosures: L. Schramm: None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR057.25/B39

Topic: A.10. Schizophrenia

Support: NIMH Grant P50MH112491
Knut och Alice Wallenberg Postdoctoral Fellowship KAW 2022.0353

Title: Elevated voltage-gated sodium channel currents and altered synaptic connectivity in an in vitro model of schizophrenia and bipolar disorder

Authors: *A. GONZALEZ RAMOS[1], E. KELLY[1], P. PALENIKOVA[1], L. CUI[1], Y.-H. HSU[1], G. PINTACUDA[1], E. KURGANOV[1], K. LAGE[2], J. Q. PAN[1], R. NEHME[1], A. J. GRANGER[1]; [1]Stanley Ctr. for Psychiatric Res., [2]Novo Nordisk Fndn. Ctr. for Genomic Mechanisms of Dis., Broad Inst. of MIT and Harvard, Cambridge, MA

Abstract: Common-variant genome-wide association studies for schizophrenia (SCZ) and bipolar disorder (BD) highlight polygenic risk but explain limited variance. Two recent exome meta-analyses -SCHEMA (n ≈ 24k cases) and BipEx (n ≈ 14k cases)- revealed ultra-rare loss-of-function (LoF) mutations in genes with odds ratios 5-50, providing high-penetrance entry points for the mechanistic study of severe psychiatric illness. Among those, *A-kinase anchoring protein 11* (*AKAP11*) stands out as a shared large-effect risk gene for both neuropsychiatric conditions. We hypothesize that loss of *AKAP11*, a central regulator of protein kinase-A (PKA), will result in alterations to neuronal excitability and synaptic development. Using CRISPR/Cas9 we introduced representative LoF mutations to the *AKAP11* gene into human embryonic stem cells generating heterozygous and homozygous isogenic cell lines; parallel CRISPR-interference *AKAP11* knock-downs (KD) were created in isogenic induced pluripotent stem cells. All lines were differentiated into excitatory cortical-like neurons (hNs) by *NGN2* overexpression and co-cultured with primary astrocytes. Whole-cell patch-clamp was used to assess basic neuronal function, observing an increase in voltage-gated sodium channel (VGSC) current and quantal synaptic properties. The increase in VGSC currents can be partially explained by an increased protein level for certain channels determined by proteomics, and preliminary alterations in the activation kinetics and long-term inactivation of the channels. Currently, we are performing high-throughput physiological VGSC measurements coupled with pharmacological experiments, as well as semi-automated high-content image analysis for a mechanistic dissection of the pathophysiological changes. Preliminary results indicate an increased somatodendritic complexity and synaptic counting deficits. These alterations in basic neuronal function may lead to further effects in network connectivity and function, which are currently being explored by calcium imaging. Modeling multiple high-penetrance LoF mutations linked to SCZ and BD in hNs has shown sizeable, directionally consistent alterations in sodium-channel activity, somatodendritic architecture, and quantal synaptic properties -phenotypes concordant with post-mortem reports of reduced synaptic density and perturbed VGSC expression in patient cortex-. Therefore, our isogenic hNs platform bridges genetic findings to cellular pathophysiology and provides a quantitative assay suitable for mechanistic dissection and therapeutic screening.

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Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

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Program #/Poster #: PSTR057.26/B40

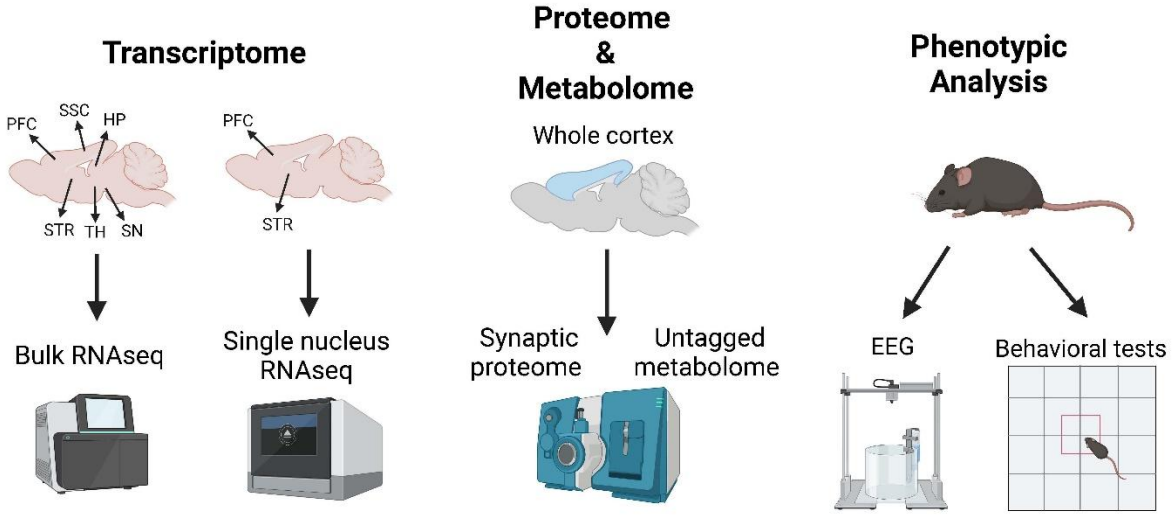
Topic: A.10. Schizophrenia

Support: Stanley Center for Psychiatric Research

Title: Epigenetic Alterations and Inhibitory Neuron Gene Deficits in Zmym2 Mutant Mice, a Genetic Model of Schizophrenia and Neurodevelopmental Disorders

Authors: *W.-C. HUANG, S. ARYAL, B. J. SONG, N. GOBLE, C. GENG, A. ASAN, X. LIU, A. HALL, Y. WANG, P. S. KUNWAR, J. Q. PAN, M. H. SHENG;
Broad Inst., Cambridge, MA

Abstract: Loss-of-function mutations in *ZMYM2* are associated with increased risk for schizophrenia (SCZ) and neurodevelopmental disorders (NDD). *ZMYM2* encodes a protein that interacts with histone-modifying and gene-regulatory complexes, yet its functions in the brain remain poorly understood. In this multi-omics study, we examined the effects of heterozygous *Zmym2* knockout in mice and identified: (1) widespread transcriptomic alterations and germline gene de-repression in the brain; (2) spatiotemporal dysregulation of diverse molecular pathways, including those related to histone modification and neuronal activity; (3) altered expression of GABAergic and interneuron-associated genes; and (4) neurophysiological and behavioral abnormalities, including changes in low-frequency EEG oscillations and locomotor hyperactivity. Together, these findings underscore the critical role of *ZMYM2* in brain development and function and support *Zmym2* mutant mice as a genetic model for SCZ and NDD.



Disclosures: W. Huang: None. **S. Aryal:** None. **B.J. Song:** None. **N. Goble:** None. **C. Geng:** None. **A. Asan:** None. **X. Liu:** None. **A. Hall:** None. **Y. Wang:** None. **P.S. Kunwar:** None. **J.Q. Pan:** None. **M.H. Sheng:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.27/B41

Topic: A.10. Schizophrenia

Support: Stanley Family Foundation

Title: Reduction of SynGAP-γ, disrupted splicing of Agap3, and oligodendrocyte deficits in Srrm2 mice, a genetic model of schizophrenia and neurodevelopmental disorder

Authors: *S. ARYAL, C. GENG, M. KWON, N. GOBLE, A. ASAN, J. Q. PAN, M. H. SHENG;
Stanley Ctr. for Psychiatric Res., Broad Inst., Cambridge, MA

Abstract: Rare loss-of-function (LoF) variants in *SRRM2*, which encodes the SRRM2 splicing factor, are associated with schizophrenia and neurodevelopmental disorder. How haploinsufficiency of *SRRM2* leads to brain dysfunction is unknown. We find that *Srrm2*$^{+/-}$ mice display (i) large-scale changes in gene expression in neuronal and glial cells, affecting synapse-, translation-. mitochondria-related molecular pathways across multiple brain regions, (ii) reduction of multiple key postsynaptic proteins, especially the gamma isoform of SynGAP, itself encoded by a neurodevelopmental disorder risk gene, (iii) abnormal splicing and elevated expression of Agap3, a SynGAP interactor, (iv) reduced numbers of oligodendrocytes accompanied by decreased expression of myelin-related mRNAs and proteins. Importantly, human iPSC-derived neurons deficient in *SRRM2* exhibit conserved AGAP3 splicing defects. Behaviorally, *Srrm2*$^{+/-}$ mice had reduced locomotor activity and impaired startle response. By EEG, *Srrm2*$^{+/-}$ mice showed reduced sleep spindles resembling humans with schizophrenia. Our findings reveal specific synaptic changes, abnormal splicing, and impaired myelination as potential mechanisms and therapeutic targets for schizophrenia and *SRRM2* LoF neurodevelopmental disorder.

Disclosures: S. Aryal: None. **M.H. Sheng:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.28/B42

Topic: A.10. Schizophrenia

Support: AZV NW25-04-00454
Charles University research program Cooperatio-Neurosciences (207038)
ERDF-Project Brain dynamics, No. CZ.02.01.01/00/22 008/0004643

Title: Gnathonemus petersii fish: a novel model of communication impairments in schizophrenia

Authors: *V. LANGOVA[1,2], I. CHRTKOVA[1,3], J. HORACEK[1,2];
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Abstract: Schizophrenia is characterized by social communication deficits that are difficult to model in traditional animal systems. We studied pharmacologically induced analogues of schizophrenia symptoms in *Gnathonemus petersii* (*G. petersii*), a novel model of schizophrenia. In particular, we examined ketamine's impacts on social behavior and electrocommunication. For *G. petersii* social studies, we developed an unsupervised algorithm using t-distributed Stochastic Neighbor Embedding (t-SNE) features of electric organ discharges (EODs) to distinguish individual fish's electric signaling. As a pilot, this study examined dyadic social interactions. Pairs consisted of one fish pre-exposed to ketamine (30 mg/L) or clear water (control), and a non-intoxicated conspecific. We used 14 juvenile ketamine-exposed fish and 3 non-intoxicated social fish. Sex was not determined as there is no dimorphism in captivity. We counted EODs from both fish and all initiated behavioral patterns, specifically agonistic, friendly, and antagonistic behaviors. We hypothesized a decrease in ketamine-exposed fish similar to negative schizophrenia symptoms. Findings show ketamine increased interactions initiated by non-intoxicated fish (p=0.02). Ketamine-exposed fish's absolute interaction number was unaffected, but relatively decreased within the pair (p=0.009). Importantly, ketamine significantly reduced agonistic behavior initiated by ketamine-exposed fish (p=0.02), similarly to some schizophrenia symptoms. Untreated fish interacting with ketamine-exposed individuals showed a trend toward increased agonistic behavior (p=0.06). These results highlight differential impacts of ketamine on social dynamics in *G. petersii* pairs and indicate the presence of analogues of negative schizophrenia symptoms. EODs were separated but unaffected by ketamine, possibly because of the electrolocation component, suggesting overall EOD number may not be a specific enough parameter. Future experiments will link specific behavioral and EOD patterns. This study confirmed ketamine's impact on *G. petersii* social behavior, analogous to schizophrenia, by reducing some behavioral patterns. Additionally, ketamine-treated fish elicited behavioral changes in non-intoxicated conspecifics. These findings suggest *G. petersii* is a valuable model for studying communication in schizophrenia and other psychiatric disorders and a basis for further studies evaluating specific relations between behavioral and EOD patterns. The usability of the algorithm for *G. petersii* social studies was confirmed and will be further implemented for studies of EOD patterns.

Disclosures: V. Langova: None. **I. Chrtkova:** None. **J. Horacek:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.29/B43

Topic: I.04. Executive Functions

Support: NIH Grant R01 MH126449

Title: Remote deep phenotyping reveals neurocognitive differences between the schizophrenia-associated 3q29 deletion and reciprocal 3q29 duplication

Authors: *R. POLLAK[1], D. BISHOP[2], M. HARNER[1], J. PURCELL[1], T. IRVING[2], K. RUPAREL[3], R. C. GUR[3], R. E. GUR[3], J. G. MULLE[1];
[1]Dept. of Psychiatry, [2]Ctr. for Advanced Biotech. and Med., Rutgers, The State Univ. of New Jersey, Piscataway, NJ; [3]Dept. of Psychiatry, Neurodevelopment and Psychosis Section, Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: Background Individuals with 3q29 deletion syndrome (3q29del) are at increased risk for neurodevelopmental and psychiatric phenotypes, including a >40-fold increased risk for schizophrenia. The comparatively understudied reciprocal 3q29 duplication syndrome (3q29dup) is associated with intellectual disability and social deficits. In similar CNV disorders neurocognitive ability is associated with psychiatric features; however, neurocognitive function has not been explored in 3q29del or 3q29dup. Here, we define the neurocognitive profile of 3q29del and 3q29dup and assess its correlation with comorbid neurodevelopmental and psychiatric phenotypes for the first time.**Methods**Individuals with 3q29del (n=70, 53% male), 3q29dup (n=22, 45% male), familial controls (n=125, 40% male), and unrelated controls (n=18, 39% male) were recruited for a deep phenotyping study where instruments were administered remotely. Participants were assessed for neurocognition (Penn Computerized Neurobehavioral Test Battery, CNB); visual-motor integration (Beery-Buktenica VMI, VMI); and neurodevelopmental and psychiatric history, including prior cognitive testing. We investigated the relationship between CNB performance and cognitive ability, visual-motor integration, and neurodevelopmental and psychiatric phenotypes in 3q29del and 3q29dup.**Results**We identified global deficits in cognitive performance in both 3q29del and 3q29dup relative to unrelated controls ($p<0.05$ across all domains). The 3q29 deletion is associated with a more substantial deficit as compared to 3q29dup. There are deficits in accuracy and efficiency in both 3q29del and 3q29dup; however, speed is relatively well-preserved in 3q29dup. Familial controls for both syndromes perform significantly worse than unrelated controls. VMI deficits in subjects with 3q29del or 3q29dup were associated with significantly worse scores on CNB tasks requiring integration of visual and motor function ($p<0.01$). CNB global accuracy scores were positively associated with IQ scores for both 3q29del and 3q29dup ($p<0.05$). **Conclusions**This is the first report of neurocognitive profiling in individuals with 3q29del and 3q29dup. We found global deficits in performance, with more significant deficits in individuals with 3q29del as compared to 3q29dup. Global accuracy scores were correlated with IQ in both 3q29del and 3q29dup, suggesting CNB performance could be a proxy for IQ in this population. Together, these results highlight the value of remote phenotyping for rare genetic disorders like 3q29del and 3q29dup and provide nuanced insight into the cognitive profiles for both disorders.

Disclosures: R. Pollak: None. **D. Bishop:** None. **M. Harner:** None. **J. Purcell:** None. **T. Irving:** None. **K. Ruparel:** None. **R.C. Gur:** None. **R.E. Gur:** None. **J.G. Mulle:** None.

Poster

PSTR057: Schizophrenia: Genetics, Pathophysiology, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR057.30/B44

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: Dr. Cynthia Shannon Weickert (PI)

Title: Transcriptional changes in perineuronal net components in schizophrenia prefrontal cortex

Authors: *J. X. CIMINO[1], C. S. WEICKERT[4,2], R. T. MATTHEWS[5], F. A. MIDDLETON[3], Y. ZHU[1];
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Abstract: Schizophrenia (SZ) is a neuropsychiatric disorder that affects 1% of the population and negatively impacts the function of the dorsolateral prefrontal cortex (DLPFC). The DLPFC is one of the last brain regions to mature, and a lack of proper maturation may precipitate symptom onset during adolescence or young adulthood. Furthermore, ~40% of SZ patients have high levels of brain inflammation that could impair DLPFC development. Reductions in both parvalbumin (PV) interneurons and density of perineuronal nets (PNNs), extracellular matrix (ECM) surrounding PV neurons, have been found in SZ brains. However, the results of quantitative PNN studies have been mixed, and molecular changes in components of the PNN have not been identified. Here we tested if SZ or inflammation status could impact mRNA levels of PNN components in human DLPFC. We examined mRNA levels for Aggrecan (ACAN), Brevican, Neurocan, Versican, Hyaluronan and Proteoglycan Link Protein 1 (HAPLN1), Tenascin-R, Contactin 1 (CNTN1), Chondroitin Sulfate Proteoglycan 5, and Receptor-Type-Tyrosine-Protein Phosphatase Zeta (PTPRZ1) using RT-qPCR. RNA was extracted from the middle frontal gyrus from controls (n = 69) and SZ patients (n = 72). We further divided the cohort into subgroups of high or low inflammation status. We found that two PNN-related mRNAs differed by diagnosis with HAPLN1 mRNA levels lower in SZ (p = 0.008), and CNTN1 mRNA levels higher in SZ (p = 0.030). When analyzed across inflammation subgroups, we found more differences in PNN transcript levels. We found higher ACAN mRNA levels in the high-inflammation SZ subgroup ($F(3, 129) = 4.415$, p = 0.005) and higher PTPRZ1 mRNA levels in both inflammation subgroups ($F(3,129) = 1\ 1.280$, p = 1.274E-06). In contrast, HAPLN1 mRNA levels were lower in the high-inflammation SZ subgroup compared to controls ($F(3, 128) = 3.826$, p = 0.012). It is unclear whether these changes are underpinning changes in PNN proteins, composition, or PNN+ cell density in SZ, as our measures focus on RNA from brain homogenates and could also include transcripts for diffuse ECM. Because HAPLN 1 is essential for ECM integrity, decreased expression could indicate a loss of ECM integrity and hyaluronic acid production that may relate to underlying inflammation. As for the increase in gene expression levels of CNTN1, and ACAN expression in SZ, this may be a compensatory mechanism aimed at rebuilding PNNs. Increased PTPRZ1 expression may indicate increased phosphocan production in SZ cortex, but protein and activity measures are needed to test this possibility. By studying these components researchers may be better positioned to devise novel treatments to restore cortical plasticity in SZ.

Disclosures: J.X. Cimino: A. Employment/Salary (full or part-time): SUNY Upstate Medical University. **C.S. Weickert:** None. **R.T. Matthews:** None. **F.A. Middleton:** None. **Y. Zhu:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.01/B45

Topic: B.03. Ion Channels

Support: NSTC 112-2314-B-002-028

Title: Disruption of human ClC-2 chloride channel proteostasis by leukodystrophy-related mutations

Authors: *C. YOU[1], A. CHENG[1], C.-J. JENG[3], C.-Y. TANG[2];
[1]Grad. Inst. of Physiol., Dept. of Physiology, Col. of Medicine, Natl. Taiwan Univ., Taipei City, Taiwan; [2]Grad. Inst. of Physiol., Dept. of Physiology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [3]Anat. and Cell Biol., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: The voltage-dependent chloride channels ClC-2 is a member of the CLC channel/transporter superfamily. Like other members of the CLC channel/transporter family, functional ClC-2 channel comprise homodimeric structures, with each monomer containing an intracellular α-helix (helix A), 17 transmembrane α-helices (helices B to R), and two cytosolic cystathionine-β-synthase (CBS) domains. ClC-2 exhibits ubiquitous expression and has been identified in both astrocytes and oligodendrocytes, where it colocalizes with the adhesion molecule GlialCAM at astrocyte-astrocyte junctions. Mutations in the human genes encoding either ClC-2 or GlialCAM have been associated with the white matter disorder leukodystrophy. The disease-associated A500V mutation in the transmembrane helix O of human ClC-2, which markedly increases side-chain volume, has been shown to cause a pronounced reduction in ClC-2 protein levels. Importantly, this alanine corresponds to one of several highly conserved residues in helix O that are found across nearly all members of the CLC channel/transporter superfamily. We therefore introduced valine substitutions at other highly conserved alanine residues in helix O to examine how these changes affect ClC-2 protein homeostasis. Biochemical data revealed that each helix O alanine mutation led to a pronounced decrease in ClC-2 total protein abundance and surface expression, and also increased its association with an E3 ubiquitin ligase implicated in endoplasmic reticulum (ER) protein quality control. Furthermore, adjusting the spatial arrangement of helices surrounding helix O can restore impaired protein stability. Collectively, these observations highlight the importance of side-chain volume at conserved helix O alanine positions in regulating ER quality control mechanisms for the human ClC-2 channel.

Disclosures: C. You: None. **A. Cheng:** None. **C. Jeng:** None. **C. Tang:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.02/Web Only

Topic: B.03. Ion Channels

Support: Beckman Scholars Program 2024

Title: Understanding the differential impact of K2P overexpression and NALCN channel knockdown on cellular membrane potential

Authors: *E. R. ELLIOTT, R. L. COOPER;
Biol., Univ. of Kentucky, Lexington, KY

Abstract: Leak channels are known to play a significant role in physiology — both in general and directly as regards the nervous system — with particular influence on excitability. Two-pore domain potassium (K2P) channels are known to affect resting membrane potential through regulation of potassium efflux. Expression of these channels is altered under certain pathological conditions — such as diseased/cancerous tissues — so it is of interest to understand the effects of altered channel expression. Three *Drosophila melanogaster* lines were observed: an experimental group (comprising an overexpression of conducting K2P channels) and two control groups (one with nonconducting K2P channels, and the other a parental line). Preparations were exposed to saline solutions containing varied concentrations of extracellular potassium (5, 0, 5, 10, 15, 25, 35, 100, 150, 180, 200, and 220 mM) and the membrane potential was measured throughout. A minimum of ten preparations were examined in each case. Parallel work was performed into the effects of altered sodium leak channel (NALCN) expression through two RNAi lines — Mid1 and narrow abdomen (*na*) — with the same protocol of extracellular potassium alteration (at the same concentrations). The results suggest that overexpression of K2P channels resulted in a significantly different response observed between the K2P line and the nonconducting line ($n = 10$; $p \leq 0.03$; t-test), while the Mid1 line was observed to be significantly different from both UAS control lines studied (given 35 mM extracellular potassium: $p = 0.012$ and 0.022 respectively; ANOVA) and the other experimental line (35 mM; $p = 0.031$; ANOVA). Overexpression of K2P channels significantly maintained a hyperpolarized state in normal ionic concentrations ($n = 10$; $p \leq 0.05$; t-test).

Disclosures: E.R. Elliott: None. **R.L. Cooper:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.03/Web Only

Topic: B.03. Ion Channels

Support: Chellgren Fellow (JKS)

Title: Classifying theDrosophiladORK1 K^+ channel: A K2P channel subtype

Authors: *J. SCHWAMBURGER[1], M. HADJISAVVA[2], R. L. COOPER[3];
[1]Univ. of Kentucky, Wheelersburg, OH; [3]Dept Biol, [2]Univ. of Kentucky, Lexington, KY

Abstract: The family of two pore domains (K2P) channels are now recognized as selective K+ ion channels that play a significant role in the resting membrane potential (RP) of cells. These are generally referred to as the K+ leak channel and allow K+ to flux based on the electrochemical gradient. These channels were 1st identified in yeast (TOK1) and subsequently the gene was identified in *Drosophila* and cloned from the neuromuscular tissues of the adult fruit fly *Drosophila melanogaster* as *KCNK0* gene) In 1996, K2PØ (also called KCNKØ, or dORK), KCNKO K+ channel follows the HUGO (Human Genome Organization) classification. Many different subtypes have now been identified and are defined by their pharmacological and biophysical properties. Some appear to be stretch activated as well as sensitive to pH while others are selective to pharmacological and anesthetic agents. Humans have 15 different K2P channel subunits (i.e., KCNK) while it is estimated that there are 11 known K2P subtypes in *Drosophila* based on genome sequencing. The expression profiles of all the subtypes in various tissues are unknown. Some cells may express a multitude of subtypes. One subtype that has been described and cloned from the *Drosophila* genome is dORK1. ORK1 expression was shown to be expressed in the larval *Drosophila* heat tube. To aid in describing the characterization of this channel we are screening various known agents which are used to define physiological subtypes. By overexpression of ORK1 in larval skeletal muscle we are comparing the agonist and antagonist known to impact various types of K2P channels in mammals. We are using known K+ channel blockers (TEA, 4-AP, barium, quinidine, ruthenium red) as well as sensitivity to external and internal pH. To date it appears TEA does not have an effect even at 20 mM ($P>0.05$ t-test). 4-AP is no effect on muscle RP ($P>0.05$ t-test) but appears to cause the motor neuron to start spontaneously firing action potentials producing synaptic responses. Thus, the dORK1 K2P channel has a unique pharmacological profile.

Disclosures: J. Schwamburger: None. **M. Hadjisavva:** None. **R.L. Cooper:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.04/B46

Topic: B.03. Ion Channels

Title: Asic1a modulates cocaine-induced locomotor activity and sensitization in a sex-specific manner

Authors: A. BADER[1], D. GLASER[1], E. CHAI[2], C. CACOULIDIS[1], ***X. CHU**[3];
[1]Univ. Of Missouri-Kansas City, Kansas City, MO; [2]Univ. of Missouri-Kansas City Sch. of Med., Chesterfield, MO; [3]Univ. of Missouri Kansas City, Kansas City, MO

Abstract: The role of acid-sensing ion channel 1a (ASIC1a) in cocaine-induced behaviors has become an area of growing interest, particularly regarding its sex-specific effects. In this study, we examined the impact of ASIC1a knockout (KO) on locomotor activity and behavioral sensitization in both male and female mice at an age of 5- to 6-month-old during acute and chronic cocaine exposure. Our findings revealed that male ASIC1a KO mice exhibited reduced locomotor activity compared to wild-type (WT) males when administered a 20 mg/kg intraperitoneal (i.p.) injection of cocaine, this difference was not statistically significant. In contrast, ASIC1a KO female mice exhibited significantly heightened locomotor activity compared to WT females on the first day of cocaine injection (20 mg/kg; i.p.). Over the course of first 3 days of cocaine (20 mg/kg; i.p.) exposure, ASIC1a KO, but not WT, females displayed significantly increased locomotor activity compared to their male counterparts. After a two-week break, both WT and ASIC1a KO male mice developed behavioral sensitization following a 10 mg/kg cocaine challenge injection (i.p.), with KO males showing significantly heightened sensitization compared to WT males. Interestingly, both WT and ASIC1a KO female mice exhibited similar behavioral sensitization to the cocaine challenge, with no significant differences between the groups. These findings underscore the differential role of ASIC1a in cocaine-induced locomotor activity and sensitization, highlighting a sex-dependent mechanism in drug-induced behaviors. Understanding the precise role of ASIC1a in addiction could provide new insights into the development of targeted, sex-specific therapies for cocaine and other substance use disorders.

Disclosures: A. Bader: None. **D. Glaser:** None. **E. Chai:** None. **C. Cacoulidis:** None. **X. Chu:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.05/B47

Topic: B.03. Ion Channels

Support: ANR
AFM
Institut Analgesia
Idex Université Côte d'Azur

Title: Modulation of ASICs by endogenous LPC analogues and potential involvement in pain

Authors: *H. LUBRANO DI SCAMPAMORTE[1], K. DELANOE[1], E. LINGUEGLIA[1], F. MARCHAND[2], E. DEVAL[1];

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Abstract: ASICs (Acid-Sensing Ion Channels) are excitatory cationic channels known to be activated by extracellular protons. They are widely expressed in the entire pathway of pain, where they participate in different processes of nociception and pain. Besides protons, endogenous lipids - such as lyso-phosphatidylcholine (LPC) - have been identified as modulators of ASICs. These lipids are therefore potentially able to alter nociceptive signalling with pathophysiological consequences, and we recently demonstrated a role of LPC in the development of rheumatic pain both in rodents and humans. LPC actually stands for a family of different ester-lipid species bearing a fatty acid chain on either the *sn1* or *sn2* position of the glycerol backbone, but it also has endogenous ether-lipid analogues. Such structural complexity raises the hypothesis that these analogues could also modulate/activate ASICs with potential consequences in painful pathologies. My research focuses on characterising some of these ether-lipid analogues regarding their effects on ASICs and their possible involvement in pain. We first used *in vitro* electrophysiological approaches to determine whether different ether-lipid analogues of LPC could activate and/or potentiate ASIC3 currents both in an heterologous expression system (HEK-293 cells) and cultured mouse dorsal root ganglia (DRG) neurons (n>10). These experiments showed that both the plasmanyl and plasmenyl-LPC can significantly modulate ASIC3, similarly to LPC. However, surprisingly, the *sn-2* LPC analogue had no effect on ASIC3 channels. To test the potential relevance of plasmanyl-LPC - the most potent lipid analogue - in muscle pain, wild-type (WT) and ASIC3-knocked-out C57/Bl6 male mice (n>10) were injected with this analogue in gastrocnemius muscle. Pain-like behaviours were assessed through Von Frey experiment, showing that the lipid could induce an ASIC3-dependant long lasting secondary mechanical allodynia. Altogether, our results indicate that endogenous lipids such as LPC and some of its ether-lipid analogues are positive modulators of ASICs and especially ASIC3, which could be important in the pathophysiology of chronic pain arising from deep tissues such as muscle or joint. For the next steps, this investigation aims to better characterise the molecular mechanisms by which lipids act on ASIC3, with perspectives towards a better understanding of nociception signalling through this particular ion channel behaving as a coincident detector, as well as of rheumatic pain pathophysiology.

Disclosures: H. Lubrano di scampamorte: None. **K. Delanoe:** None. **E. Lingueglia:** None. **F. Marchand:** None. **E. Deval:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.06/B48

Topic: B.03. Ion Channels

Title: A Novel Negative Allosteric Modulator of NMDA Receptor with Antidepressant Efficacy

Authors: *J. WANG, S. ZHU;
Ctr. for Excellence in Brain Sci. and Intelligence Technol., Shanghai, China

Abstract: Depression is currently the most prevalent mental disorder, affecting approximately 2 billion people worldwide, making it a global public health concern. Ketamine is a leading antidepressant drug that primarily targets NMDA receptors as a channel blocker. However, ketamine carries potential risks such as hallucinogenic effects, dissociation, and addiction. Using the two-electrode voltage-clamp technique, we conducted a large-scale screening of small molecules modulating NMDA receptor currents in Xenopus oocytes and identified a series of compounds, among which P3D11 selectively inhibited NMDA receptor currents with an IC50 of approximately 1.48 μM. Unlike ketamine, P3D11 does not suppress NMDA receptor currents via channel blockade but rather through a negative allosteric modulation mechanism. In in vivo studies, we found that intraperitoneal injection of P3D11 induced a long-lasting antidepressant phenotype in mice. The discovery of this small molecule holds significant implications for elucidating the biophysical properties of NMDA receptors and the NMDA receptor-related mechanisms underlying antidepressant effects.

Disclosures: J. Wang: None. **S. Zhu:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.07/Web Only

Topic: B.03. Ion Channels

Title: High-throughput electrophysiology and fluorescence-based screening platforms for the identification of activators of the lysosomal ion channel TMEM175

Authors: R. BURLEY[1], A. BROOK[2], J. BETTS[2], X. KODJI[2], R. ABDELAZIZ[2], S. STABILINI[2], K. KUAN[2], *A. K. CHOUHAN[3], T. ROBERTS[2];
[1]Early Discovery, [2]Biol., [3]Small Molecule Drug Discovery, Charles River Labs., Saffron Walden, United Kingdom

Abstract: TMEM175 created a surge of interest across the scientific community when it was identified that there was a clear association between genetic variation at this gene locus and development of Parkinson's Disease (PD). Initially characterized as a lysosomal potassium conductance it was demonstrated to have a role influencing lysosomal function by maintaining intra-lysosomal pH. Since lysosomes contribute to the clearance and degradation of α-synuclein, loss of function is implicated in a more rapid appearance and build-up of the pathological toxic aggregates associated with the symptoms of PD. Genetic studies link TMEM175 variants to increased disease risk and accelerated progression of symptoms, highlighting its potential as a therapeutic target.High-throughput screening (HTS) assays are essential for identifying activators of TMEM175. Developing robust HTS assays enables the rapid identification of small-molecule

activators, accelerating drug discovery efforts to study their role in modulating lysosomal clearance. By unlocking TMEM175's therapeutic potential, HTS-driven approaches offer a promising path towards disease-modifying treatments for PD.The work describes the development of fluorescence-based, thallium flux screening assays and automated electrophysiology using the Sophion Qube for the measurement of outward ion channel currents carried by Cs+, and inward proton currents. Assay metrics and pharmacology data profiling the activity of tool activator compounds show the conditions established to measure single-point screening data and full concentration-response data necessary for profiling compounds to establish potency. The assays are robust and scalable to allow for their use in HTS. Data are compared using HEK293 cell lines overexpressing the human wild-type channel, the human M393T loss of function variant, the human gain of function Q65P variant, and a mouse wild-type TMEM175 channel.The data support the implementation of this methodology as a primary screening assay in a hit identification cascade suitable for identifying novel activators of TMEM175 suitable for drug discovery.

Disclosures: R. Burley: None. **A. Brook:** None. **J. Betts:** None. **X. Kodji:** None. **R. Abdelaziz:** None. **S. Stabilini:** None. **K. Kuan:** None. **A.K. Chouhan:** None. **T. Roberts:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.08/B49

Topic: B.03. Ion Channels

Support: Fonds de la Recherche Scientifique – FNRS Grant ASP-REN40024838
Belgian Government through the Federal Public Service Policy and Support Grant NEMODEI

Title: Slow pacemaking dynamics from fast activating kinetics in midbrain dopamine neurons

Authors: *A. FYON[1,2], O. PAVLOVA[3], S. RINGLET[4], A. FRANCI[2,5], N. SCHAAR[6], J. ROEPER[6], V. M. SEUTIN[3], G. DRION[2], K. JEHASSE[2];
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Abstract: Midbrain dopamine neurons exhibit slow and regular pacemaking supporting tonic dopamine release that is essential for motor and cognitive functions. The biophysical origin of this slow rhythmicity remains debated. To sustain such slow frequency of firing, a small amplitude current is needed during the interspike interval. It could originate from either cooperative ion channels or very small conductance pores. Recent work showed that the

pacemaker current does not result from cooperative channels, but from a newly identified small conductance pore sensitive to the gating-pore blocker 1-(2,4-xylyl)guanidinium (Jehasse et al, 2021). This conductance is considered as a pacemaker conductance (g_{pace}) as it is active during the interspike interval and its blockade silences the spontaneous activity of midbrain dopamine neurons. However, its molecular identity and kinetics remains to be determined.
We modeled this conductance from the voltage clamp data of Jehasse et al. and included it in a conductance-based model of dopamine neurons (Yu & Canavier, 2015) to investigate its biophysical properties. We first adjusted activation kinetics of sodium and calcium conductances to physiological values and observed that, without g_{pace}, the model was not able to generate spontaneous activity nor slow frequency firing. Moreover, g_{pace} restored slow pacemaking over a wider parameter space, even when conventional mechanisms failed. Our simulations reveal that only transient sodium, delayed rectifier potassium channels and g_{pace} are required to produce slow pacemaking. Our findings suggest that slow-frequency firing requires the activation kinetics to be extremely fast. We also explored the role of other currents (e.g., A-type, H, and SK) and observed that they mainly enhance robustness to synaptic noise by reducing spike time variability, as observed before experimentally (Higgs et al., 2023).
This mechanism, based on fast activation driving slow rhythmicity, contrasts with previous theories emphasizing slow-inactivating conductances (Bean, 2024). Our work introduces a minimal and robust framework for an alternative mechanism of slow pacemaking and lays the groundwork for dynamic clamp experiments and molecular identification of this novel current.
Bean, B. P. (2024). *The Journal of Physiology.*
Higgs, M. et al. (2023). *eNeuro*, 10
Jehasse, K., et al. (2021). *Neuropharmacology*, 197, 108722.
Yu, N., & Canavier, C. C. (2015). *The Journal of Mathematical Neuroscience (JMN)*, 5, 1-19.

Disclosures: A. Fyon: None. **O. Pavlova:** None. **S. Ringlet:** None. **A. Franci:** None. **N. Schaar:** None. **J. Roeper:** None. **V.M. Seutin:** None. **G. Drion:** None. **K. Jehasse:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.09/B50

Topic: B.03. Ion Channels

Title: Mechanisms of DRG sensitization in neuropathic pain after spinal cord injury

Authors: *M. CHEN;
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Abstract: Neuropathic pain is a significant consequence of spinal cord injury (SCI) that drastically impairs the quality of life for SCI victims more significantly than catastrophic motor deficits. Current treatments for pain offer poor analgesic effects with serious side effects, highlighting the urgent need for novel therapies to deter the onset of intractable chronic pain.

Following SCI, neurons in the dorsal root ganglia (DRG) are the main drivers of pain. DRG neurons become hypersensitive and hyperactive, which increases sensory neuron excitability and firing, causing more pain signals to be relayed to the brain. However, the molecular mechanisms of post SCI DRG neuron hyperactivity are poorly understood. SCI is classified into: primary injury, initial damage from physical forces, or secondary injury, damage due to chronic inflammation and oxidative stress that originate from primary injury. Existing studies suggest that acrolein, a toxic aldehyde and product of oxidative stress and a known key pathological factor in secondary SCI, plays a key role in promoting pain by increasing the expression and activation of TRPA1, a pain sensory channel expressed by a significant proportion of DRG neurons. However, the mechanisms of acrolein-mediated hyperactivity of TRPA1 remain unclear. Uncovering these mechanisms could better our understanding of the acrolein-mediated analgesic process and thereby provide critical insight for the establishment of novel treatment to effectively manage pain.In this study, SCI was administered *via* an impact hammer applied to thoracic vertebra 10 (T10) of male Sprague Dawley® rats. DRG cells were isolated 24 hours after SCI. Immunocytochemistry for acrolein adducts and TRPA1 was performed to quantify the presence of acrolein and TRPA1. Additionally, whole cell patch clamp was used to measure the electrical properties of individual DRG neurons, action potentials, and currents in the presence and absence of acrolein and TRPA1 antagonists and agonists in order to assess the contribution of each molecule to changes in DRG signal properties. Acrolein and TRPA1 both affected DRG electrophysiology, signaling and response to stimulus. These findings demonstrate that this combined *in vivo* and *in vitro* experimental system offers a versatile platform to study single-cell effects of SCI. Additionally, these findings quantify the extent to which TRPA1 contributes to acrolein-mediated neuropathic pain, the first step in uncovering the complex network of interactions between ion channels, secondary injury, and pain signals, providing new insights into the prevention and treatment of secondary SCI.

Disclosures: M. chen: None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.10/B51

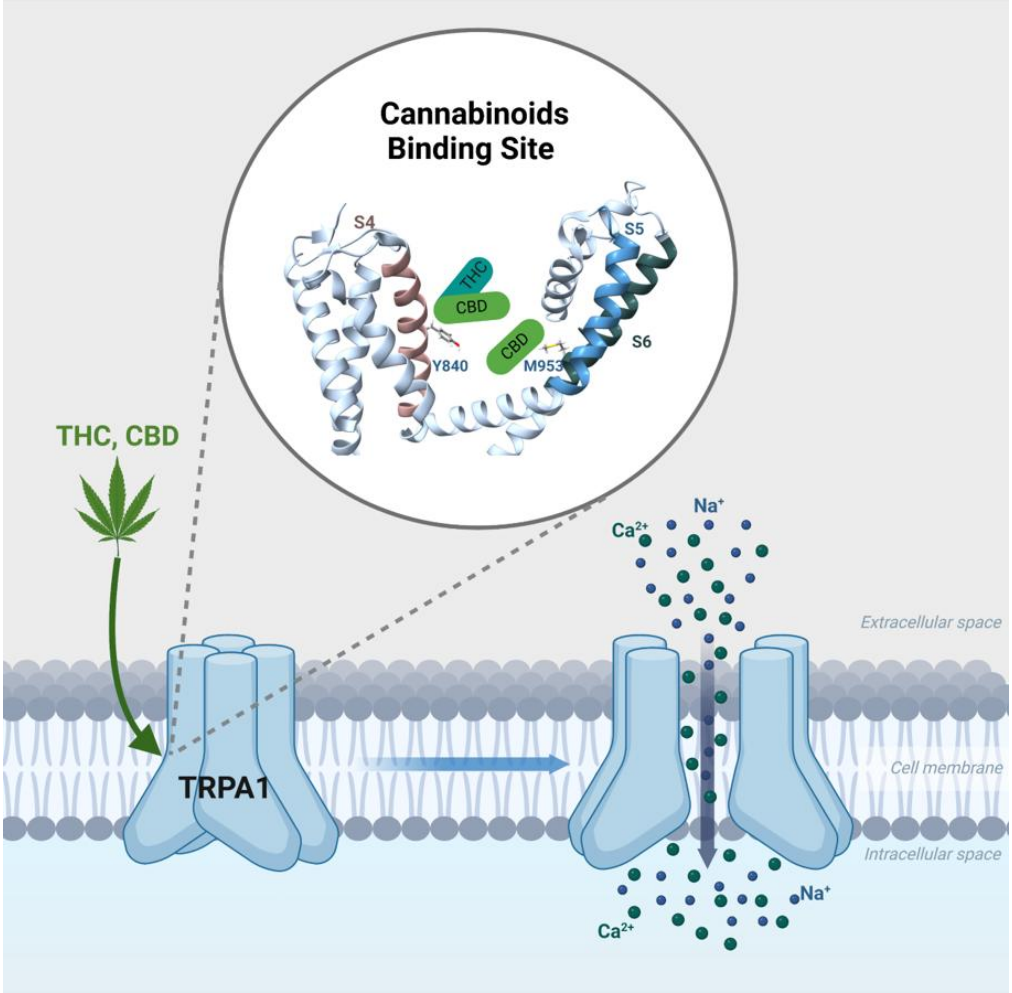
Topic: B.03. Ion Channels

Title: Identification of the TRPA1 cannabinoid-binding site

Authors: *T. AMAWI;
The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: Chronic pain accounts for nearly two-thirds of conditions eligible for medical cannabis licenses, yet the mechanisms underlying cannabis-induced analgesia remain poorly understood. The principal phytocannabinoids, the psychoactive Δ9-tetrahydrocannabinol (THC) and non-psychoactive cannabidiol (CBD), exhibit comparable efficacy in pain management.

Notably, THC functions as an agonist of cannabinoid receptor 1 (CB1), whereas CBD shows minimal activity on CB1 and CB2 receptors. Elucidating the molecular targets through which phytocannabinoids modulate the pain system is required for advancing our understanding of the pain pathway and optimizing medical cannabis therapies. Transient receptor potential ankyrin 1 (TRPA1), a pivotal chemosensor in the pain pathway, has been identified as a phytocannabinoid target. Unlike most TRPA1 activators, phytocannabinoid activation is not mediated through the electrophilic binding site, suggesting an alternative mechanism. Here, we identified the human TRPA1 channel cannabinoid-binding site (CBS) and demonstrated that mutations at residue Y840 abolished responses to both THC and CBD at saturating concentrations, indicating a shared primary binding site. Molecular modeling revealed distinct interactions of THC and CBD with the Y840 residue within the CBS. Additionally, CBD binds to the adjacent general anesthetic binding site at oversaturating concentrations. Our findings define the CBS of TRPA1 as overlapping with and adjacent to binding sites for other allosteric activators, suggesting that TRPA1 possesses a highly adaptable domain for binding non-electrophilic activators. This underscores its unique role as a chemosensor in the pain pathway. Furthermore, our results provide new insights into the molecular mechanisms of cannabinoid-induced analgesia and identify novel targets for pain management therapies.



Disclosures: T. Amawi: None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.11/B52

Topic: B.03. Ion Channels

Title: Mechanistic investigation of TRPA1 inhibition: insights from structural and functional studies with AMG9090

Authors: *M. GHANTOUS[1], T. AMAWI[2], A. PRIEL[2];
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Abstract: The transient receptor potential ankyrin 1 (TRPA1) ion channel plays a vital role in nociception, primarily in chemosensation, by detecting a wide range of chemical irritants and inflammatory mediators. While the mechanisms underlying TRPA1 activation by both electrophilic and non-electrophilic agonists are well studied, far less is known about how the channel is inhibited. Elucidating the mechanisms of human TRPA1 (hTRPA1) inhibition is essential for deepening our understanding of pain signaling and for guiding the development of selective analgesics.This project focuses on AMG9090, a potent non-electrophilic antagonist of hTRPA1. To investigate its mechanism of action, we apply a multidisciplinary strategy integrating calcium imaging, whole-cell patch-clamp electrophysiology, site-directed mutagenesis, and molecular modeling. Guided by structural docking, we introduce targeted mutations at residues predicted to contribute to AMG9090 binding. These studies aim to define the antagonist binding pocket and evaluate its overlap with previously identified allosteric sites.To examine how individual subunits contribute to TRPA1 inhibition, we engineer concatemeric constructs composed of defined combinations of wild-type and AMG9090-insensitive subunits. This approach allows us to assess the stoichiometric requirements for functional inhibition and to determine whether subunit occupancy is cooperative or independent.By identifying key binding sites and dissecting inhibitory interactions at the molecular level, we aim to advance our understanding of TRPA1's role in pain signaling and support the development of more specific and effective TRPA1-targeted analgesics.

Disclosures: M. Ghantous: None. **T. Amawi:** None. **A. Priel:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.12/B53

Topic: B.03. Ion Channels

Title: Contribution of the TRPC4 channel to elevated plus maze behavior in rats

Authors: *K. R. ILLIG[1], C. MCGLYNN[2,1];
[1]Univ. of St. Thomas, Saint Paul, MN; [2]Fenton, MO.

Abstract: Rats lacking the canonical transient receptor potential channel (TRPC) *trpc4* gene display a reduction in cocaine self-administration, without displaying a reduction in learning or performance for natural rewards (Klipec et al. 2016, *Behavioural Brain Research*). We have hypothesized that this results from the relative lack of dopaminergic cells in the VTA that exhibit high spontaneous activity in in *trpc4* knockout (KO) animals. This pathway is part of a larger circuit--including the nucleus accumbens, the hippocampal formation, and the medial prefrontal cortex--implicated in cocaine-seeking behavior. In the human population, substance abuse disorder co-occurs with anxiety at a greater rate than would be expected by chance, suggesting overlapping circuit mechanisms. We compared the behavior of *trpc4* KO animals with wild-type (WT) and heterozygous littermates as they completed their first five-minute exploration of an elevated plus maze, to determine whether removal of this channel may alter anxiety-related behavior. Forty-five rats from five litters were evaluated for overall locomotor behavior, number of open- and closed-arm entries, and overall time spent in open and closed arms of the elevated plus maze. Results revealed significant differences in *trpc4* KO animals compared to littermate WT controls. These results suggest that changes in neuronal excitability associated with the TRPC4 channel may modulate some aspects of anxiety. These results further suggest a link between the neural mechanisms underlying anxiety and addiction.

Disclosures: K.R. Illig: None. **C. McGlynn:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.13/B54

Topic: B.03. Ion Channels

Support: NIH Grant R01 NS111943
NIH Grant R21 NS140782

Title: Targeted deletion of the anion channel protein LRRC8A in GABAergic neurons modifies inhibitory signaling and animal behavior

Authors: *M. L. CHANDLER, J. W. NALWALK, A. SPRAGUE, A. A. MONGIN;
Neurosci. and Exptl. Therapeut., Albany Med. Col., Albany, NY

Abstract: Volume-regulated anion channels (VRACs) are ubiquitously expressed chloride channels that are composed of proteins from the leucine-rich repeat-containing family 8 (LRRC8A-E). While classically recognized for their role in cell volume regulation, VRACs are increasingly associated with many other physiological processes, including the modulation of

brain excitability. Human mutations associated with either the loss or gain of VRAC function result in severe neurological phenotypes. Although the underlying mechanisms remain unknown, changes in VRAC-mediated release of amino acid neurotransmitters, such as glutamate and GABA, may be responsible. To explore the significance of VRACs in the brain, our laboratory previously generated a mouse with brain-wide deletion of the essential VRAC subunit LRRC8A using *Nestin*^{Cre}-driven recombination of floxed *Lrrc8a*. Brain-specific LRRC8A knockout (KO) led to the development of spontaneous seizures and 100% mortality between 5-12 weeks of age, and this lethal phenotype was associated with modified GABAergic transmission (Wilson et al., FASEB J., 2021). In the present study, we tested the hypothesis that the loss of LRRC8A/VRAC in GABAergic cells dysregulates inhibitory signaling and prompts pathological hyperexcitability in the brain. We produced a GABAergic neuron-specific deletion of LRRC8A using *Gad2*-IRES^{Cre} (inLRRC8A KO). The specificity of Cre-targeting was validated using the Ai9 tdTomato reporter, which showed labeling throughout the brain with the highest density of Cre-targeted cells in the striatum, where inhibitory medium spiny neurons comprise ~95% of the neuronal population. The loss of LRRC8A was confirmed by western blotting. Similar to the brain-wide knockout, inLRRC8A KO mice exhibited a pronounced hyperlocomotive phenotype. However, they remained fully viable and did not show overt seizures. Molecular analysis of striatal tissue in inLRRC8A KO revealed a ~25% reduction in protein levels of the GABA transporter GAT-1 and 40-50% decreases in mRNA expression levels of both primarily synaptic (α1 and γ2) and predominantly extrasynaptic (α4 and δ) GABA(A)-R subunits. These changes were milder in brain regions with fewer Cre-targeted cells. Overall, our present work demonstrates that the targeted deletion of LRRC8A in GAD2+ neurons is sufficient to elicit significant biochemical and behavioral changes. These findings advance our understanding of VRAC function and dysfunction in the brain, including in neurological disorders.

Disclosures: M.L. Chandler: None. **J.W. Nalwalk:** None. **A. Sprague:** None. **A.A. Mongin:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.14/B55

Topic: B.03. Ion Channels

Title: Characterization of HCN Current in Human Dorsal Root Ganglion Neurons

Authors: *I. J. OROZCO[1], Y. MIRON[2], Y. SHI[2], T. COTTA[1], J.-M. PACLEB[1], F. CHEN[3], J. LIAW[3], G. PAGE[3], N. ABI GERGES[1], R. KONDO[1], A. GHETTI[1], K. CARLIN[1]; [1]Neurosci., [3]Neuorscience, [2]AnaBios, San Diego, CA

Abstract: The hyperpolarized-activated cyclic nucleotide-gated (HCN) protein is a tetrameric cation conducting ion channel. While HCN can be partially open at resting voltages, where it imparts a depolarizing current, full activation requires membrane hyperpolarization. HCN is also

positively modulated by the second messenger molecule cyclic adenosine monophosphate (cAMP). HCN has notable roles in excitable tissues of the heart and nervous system where it can modulate action potential firing. We investigated the role of HCN in human somatosensory DRG neurons which play an important role in pain perception. Using patch-clamp analysis we found that a subset of DRG neurons exhibited a prominent hyperpolarization sag, which was abolished in the presence of the HCN inhibitor ZD7288. In the presence of ZD7288, the resting membrane potential hyperpolarized by up to 10% and was associated with an increase in rheobase. Experiments combining calcium imaging and electric field stimulation in these same neurons also revealed reduced neuronal excitability in the presence of ZD7288. Overall, these results suggest that HCN plays an important role in setting the resting membrane potential and excitability of human DRG neurons and could therefore be a viable target for treating pain.

Disclosures: I.J. Orozco: None. **Y. Miron:** None. **Y. Shi:** None. **T. Cotta:** None. **J. Pacleb:** None. **F. Chen:** None. **J. Liaw:** None. **G. Page:** None. **N. Abi Gerges:** None. **R. Kondo:** None. **A. Ghetti:** None. **K. Carlin:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.15/B56

Topic: B.03. Ion Channels

Support: Brain Research Foundation
BYU College of Life Sciences
Sayers

Title: Spreading depolarizations in an ex vivo zebrafish model are preserved despite multi-channel inhibition: a comparative study with rodent slices

Authors: *P. C. WITHERS[1], K. AFRAN-OKESE[1], J. NORBY[1], A. SULI[2], R. PARRISH[1]; [2]Cell Biol. and Physiol., [1]Brigham Young Univ., Provo, UT

Abstract: Spreading depolarization (SD) is a wave of profound cellular depolarization that propagates across tissue in the central nervous system, resulting in the near-complete collapse of ionic gradients. Implicated in neuropathologies such as seizures, migraine with aura, traumatic brain injury, and stroke, SD is experimentally induced in animal models through methods including electrical stimulation, mechanical injury, hypoxia, and elevated extracellular potassium. Despite considerable attention in research, the exact mechanisms underlying SD initiation remain poorly understood. Prior research in rat models found that the simultaneous pharmacological block of sodium, calcium, and glutamatergic (AMPA and NMDA) channels completely prevents SD induction whereas inhibiting any two of these three currents is insufficient to block SD. Despite these findings, researchers have still speculated that there may be other unknown channels that contribute to SD induction. While there exists a dense repertoire

of experiments with rodent models, there has been limited SD research in other animal models, such as zebrafish. Genetically tractable and capable of affordable high-throughput screening, zebrafish have emerged as powerful vertebrate models for studying ion channel dynamics and offer a promising but underutilized model for uncovering the molecular basis of SD. Using local KCl microinjection and DC local field potential recordings, we developed and tested a novel ex vivo protocol for studying SDs in the adult zebrafish optic tectum, characterizing SD initiation after the addition of TTX, MK-801 (or APV), NBQX, and $NiCl_2$. We also conducted comparable pharmacological experiments with a similar protocol in the CA1 of submerged hippocampal mouse slices and found that, in both models, although SD amplitude decreased, SDs persisted despite the combined inhibition of sodium, calcium, and glutamatergic channels, with no significant reduction in SD duration. These findings suggest that additional mechanisms beyond sodium, calcium, and glutamatergic signaling contribute to SD induction, lending additional evidence to the existence of other potential critical contributors to SD physiology, which we are actively exploring.

Disclosures: P.C. Withers: None. **K. Afran-Okese:** None. **J. Norby:** None. **A. Suli:** None. **R. Parrish:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.16/B57

Topic: B.03. Ion Channels

Title: Characterization of human iPSC-derived RealDRGx^{TM}sensory neurons using automated electrophysiology

Authors: *S. RICE, E. BLACK, K. ARTHUR, S. GAMBRELL, E. MACMILLAN, R. FINOCCHIARO, D. DALRYMPLE, I. MCPHEE, D. PAU;
Sygnature Discovery, Glasgow, United Kingdom

Abstract: Dorsal root ganglion cells (DRG) are specialized peripheral sensory neurons known as nociceptors and a fundamental component of the human sensory pathway and pain recognition. DRGs are activated by a variety of sensory stimuli such as extreme temperature, pH, mechanical pressure and injury-related chemicals, relaying these stimuli to the Central Nervous System (CNS). Historically, due to the difficulty in sourcing human primary neurons, they have posed significant challenges with respect to higher throughput automated electrophysiology platforms, greatly limiting their use in the hit identification phase of the drug discovery process.
The recent development of human induced pluripotent stem cell (hiPSC)-derived sensory neurons displaying typical DRG electrophysiological properties, offers a valuable alternative tool for pain research, with their increasing availability enabling greater electrophysiological output. Using the high-throughput automated SyncroPatch384i electrophysiological platform (Nanion, Germany), we characterised hiPSC-derived RealDRGx^{TM} nociceptors (Anatomic, USA),

examining the electrophysiological activity of various ion channels over a 6-week period of maturation.
Using a combination of specific voltage protocols and pharmacological tools, we successfully recorded ligand-gated (GABA & Glycine) and voltage-gated (Na_V & K_V) currents. We also demonstrate the ability to identify and measure TTX-$_R$ Na_V currents, with an expected shift in voltage-dependence upon application of 500 nM TTX, suggesting expression of TTX-$_R$ subtypes which are typical in DRG cells. In addition, intrinsic neuronal excitability was characterized in current-clamp mode by assessing parameters such as resting membrane potential, spontaneous and evoked action potential generation and tracking minimum firing thresholds.
Combining RealDRGx™ and automated electrophysiology, we have shown the potential to accelerate the identification and characterisation of novel pharmacological tools for inflammatory and neuropathic pain drug discovery.

Disclosures: S. Rice: None. **E. Black:** None. **K. Arthur:** None. **S. Gambrell:** None. **E. MacMillan:** None. **R. Finocchiaro:** None. **D. Dalrymple:** None. **I. McPhee:** None. **D. Pau:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.17/B58

Topic: B.03. Ion Channels

Title: Characterization of KCNT1 using high throughput automated patch clamp: activation by internal Na

Authors: T. A. GOETZE[1], **A. R. OBERGRUSSBERGER**[1], E. BRAKSATOR[2], S. HEBEISEN[2], D. KONRAD[2], T. STRASSMAIER[3], Y.-L. LU[3], *E. DRAGICEVIC[1], N. FERTIG[1];
[1]Nanion Technologies GmbH, Munich, Germany; [2]B'SYS GmbH, Witterswil, Switzerland; [3]Nanion Technologies Inc., Livingston, NJ

Abstract: KCNT1 encodes a sodium-activated potassium channel which plays an important role in neuronal excitability. Mutations in KCNT1 have been implicated in epilepsy and neurodevelopmental disorders including epilepsy of infancy with migrating focal seizures (EIMFS) and developmental and epileptic encephalopathies (DEE). KCNT1 is activated on the intracellular side by sodium which can be challenging to achieve using conventional patch clamp techniques. We describe activation of KCNT1 stably expressed in a CHO cell line by addition of internal Na^+ via continuous perfusion of the internal solution on a high throughput automated patch clamp (APC) system. After a period of assay development, the optimal conditions for recording KCNT1 currents were established. This included activating the current by perfusing Na^+ in the internal solution after establishing the whole cell configuration. In this way, we could record KCNT1 for over 10 minutes with rundown as low as 2.4%. We investigated the

pharmacology of KCNT1 and found that KCNT1 was blocked by quinidine with an IC_{50} of approximately 130 μM and bepridil with an IC_{50} of 3 μM. Since KCNT1 is a potential target for the treatment of rare epilepsy disorders, reliable, high throughput methods for drug discovery are needed to study KCNT1 pharmacology using a stable cell line in combination with high throughput APC.

Disclosures: T.A. Goetze: A. Employment/Salary (full or part-time): Nanion Technologies GmbH, Munich, Germany. **A.R. Obergrussberger:** A. Employment/Salary (full or part-time): Nanion Technologies GmbH, Munich, Germany. **E. Braksator:** A. Employment/Salary (full or part-time): B'SYS GmbH, Witterswil, Switzerland. **S. Hebeisen:** A. Employment/Salary (full or part-time): B'SYS GmbH, Witterswil, Switzerland. **D. Konrad:** A. Employment/Salary (full or part-time): B'SYS GmbH, Witterswil, Switzerland. **T. Strassmaier:** A. Employment/Salary (full or part-time): Nanion Technologies Inc., Livingston, NJ, USA. **Y. Lu:** A. Employment/Salary (full or part-time): Nanion Technologies, Inc., Livingston, NJ, United States. **E. Dragicevic:** A. Employment/Salary (full or part-time): Nanion Technologies GmbH, Munich, Germany. **N. Fertig:** A. Employment/Salary (full or part-time): Nanion Technologies GmbH, Munich, Germany.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.18/B59

Topic: B.03. Ion Channels

Title: Cold activation of TRPM8 using high throughput automated patch clamp

Authors: M. ROTORDAM[1], J. RATHJE[1], N. BECKER[1], **A. R. OBERGRUSSBERGER**[1], *Y.-L. LU[2], T. STRASSMAIER[2], N. FERTIG[1];
[1]Nanion Technologies GmbH, Munich, Germany; [2]Nanion Technologies, Inc., Livingston, NJ

Abstract: Transient Receptor Potential (TRP) channels are widely distributed throughout the mammalian central and peripheral nervous systems. They can be directly activated by ligands, heat or cold and mechano-stimulation, and are important targets in drug discovery for the treatment of pain, respiratory diseases, cancer, immune disorders and others. TRPM8 is activated by the ligands menthol, icilin and eucalyptol, and also by low temperatures, below 25°C. TRPM8 has been implicated to play a role in chronic and neuropathic pain and is a potential target in drug discovery, therefore, it is essential to have reliable methods for recording TRPM8 activated using different stimuli to find TRPM8 modulators. Here, we activated TRPM8 using cold solution on three different automated patch clamp (APC) systems. On the SyncroPatch 384, a fully automated system for recording up to 384 wells simultaneously, continuous cooling of the recording chamber to temperatures down to 10-12°C were used to activate TRPM8 stably expressed in CHO cells. Either a voltage ramp or a voltage step protocol was used and large currents were recorded when the recording chamber and solutions were cooled down to 10 -

12°C. This activation was reversed when the recording chamber was heated to 30°C. Progressive cooling of the module and solutions led to increasing current density ranging from 20 pA/pF at 25°C up to 120 pA/pF at 10°C. What is more, using the Patchliner, solutions could be pre-cooled and rapidly applied to the cells for rapid and transient activation of cold-activated TRPM8-mediated currents. Using the Port-a-Patch, a semi-automated patch clamp system for recording 1 cell at a time, TRPM8 was repetitively activated by cold solution at 10°C using the temperature-controlled perfusion system. Capsazepine (10 μM) was used to block the activated current. Alternatively, the ligand menthol could be used to activate TRPM8 on the different APC devices. In this way, we could activate TRPM8 using different stimuli, including continuous or transient cooling to temperatures <12°C and this method could be used for high throughput screening of TRPM8 modulators for potential pain therapeutics.

Disclosures: **M. Rotordam:** A. Employment/Salary (full or part-time): Nanion Technologies. **J. Rathje:** A. Employment/Salary (full or part-time): Nanion Technologies. **N. Becker:** A. Employment/Salary (full or part-time): Nanion Technologies. **A.R. Obergrussberger:** A. Employment/Salary (full or part-time): Nanion Technologies. **Y. Lu:** A. Employment/Salary (full or part-time): Nanion Technologies, Inc. **T. Strassmaier:** A. Employment/Salary (full or part-time): Nanion Technologies, Inc. **N. Fertig:** A. Employment/Salary (full or part-time): Nanion Technologies.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.19/B60

Topic: B.03. Ion Channels

Support: NS003134

Title: Ionic mechanisms driving spontaneous activity and G-protein coupled receptor activation of locus coeruleus neurons

Authors: *N. L. O'PREY[1], Z. M. KHALIQ[2];
[1]NINDS, [2]NIH, Bethesda, MD

Abstract: The locus coeruleus (LC) is a bilateral nucleus in the dorsal pons and the brain's primary site of noradrenaline (NA)-releasing neurons. The LC is one of the first regions to degenerate with aging and in Parkinson's disease (PD). Selective degeneration in LC neurons undergo has been suggested to occur as a result of their spontaneous firing activity, which occurs at low rates (0.5-10 Hz). Thus, better knowledge of the LC neurons' electrophysiological properties may be useful to better understand their degeneration in PD. Among the mechanisms that contribute to spontaneous activity in other brain regions, the non-selective sodium leak channel NALCN functions to depolarize the resting membrane potential and drives tonic action potential. Here, we tested the hypothesis that NALCN contributes to spontaneous activity in LC

neurons and that NALCN underlies an increase in activity following activation of Gq-coupled protein receptors. To do this, we used mice in which GFP expression is driven by the tyrosine-hydroxylase (TH) promoter, a precursor for NA synthesis. We conducted whole-cell patch-clamp recordings on LC-NA neurons to analyze action potential firing properties. We found that lowering extracellular calcium from 2 mM to 0.1 mM increased LC neuron excitability and substantially increased firing. We also tested the effects gadolinium, an inhibitor of NALCN channels, has on LC firing. In future experiments, we will examine NALCN's role in Gq-coupled receptor-mediated activation of LC neurons. Illuminating NALCN's role in LC firing may inform future investigations that focus on understanding the loss of LC neurons' role in PD.

Disclosures: N.L. O'Prey: None. **Z.M. Khaliq:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.20/C1

Topic: B.03. Ion Channels

Support: CAPES/88887699287/2022-00

Title: Modulation of Feeding Behavior by K_{ATP} Channels in Serotonergic Neurons of the Dorsal Raphe Nucleus

Authors: *P. S. Z. B. ZARPELLON BARBOSA[1,3], L. L. K. ELIAS[3,2], R. M. LEAO[4,2]; [1]Physiol., Faculdade de Medicina de Ribeirão Preto - USP, Ribeirão Preto, Brazil; [2]Physiol., Faculdade de Medicina de Ribeirão Preto - USP, Ribeirao Preto, Brazil; [3]Physiol., [4]Fisiologia, Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: ATP-sensitive potassium (K_{ATP}) channels play a crucial role in coupling metabolic status and neuronal excitability in energetic control and feeding behavior centers. The Dorsal raphe nucleus (DRN) expresses serotonergic (5HT). Evidence shows that 5HT in the DRN participates in the control of feeding behavior. If these neurons express K_{ATP} channels, it is possible that K_{ATP} participates in food intake behavior. To investigate the expression and role of K_{ATP} channels in serotonergic and non-5HT neurons and how modulation of these channels can affect feeding behavior, we employed a combination of Whole-Cell Patch-Clamp technique, immunofluorescence for TpH2 (tryptophan hydroxylase type 2) and Kir6.2 (forming subunit of the K_{ATP} channel), and food intake experiments in DRN cannulated C57BL/6J mice and chemogenetics approach in SERT-Cre mice, fasted 12h and satiated animals received tolbutamide and diazoxide respectively, and fasted 12h animals received CNO or DMSO in chemogenetics experiments (CEUA 1068/2022). Differentiation between 5HT and non-5HT neurons was based on the presence of a "shoulder" during the repolarization phase of the action potential. K_{ATP} activation was induced with diazoxide, while inhibition was achieved using tolbutamide. We observed that 5HT neurons exhibited a shoulder in the repolarization phase of

the action potential, distinguishing them from non-5HT neurons (5HT neurons = 23 and animals = 10, non-5HT neurons = 12 and animals = 6). K_{ATP} expression was confirmed by immunofluorescence, TpH2+ neurons expressing Kir6.2 (n = 3 animals). Application of diazoxide to 5HT neurons caused hyperpolarization, reduced membrane input resistance, and increased membrane current (mean difference ± SEM was equal to -2.850 ± 0.335 mV, -97.30 ± 27,49 MOhm, 44.33 ± 11.08 pA at -45 mV, p value equal to 0.003, 0.002, 0.0006, respectively. n = 23 neurons, Paired t-Test), effects not observed in non-5HT neurons (n = 12 neurons). Chemogenetic activation of 5HT neurons decreased the food intake in fasted mice (-0.73 ± 0.30 g, p = 0.04, Paired t-Test). Intracranial administration of tolbutamide reduced food intake in the fasting state, while diazoxide increased food consumption in satiated animals (mean difference ± SEM was equal to -0.33 ± 0.13 g, 0.24 ± 0.04 g, p value equal to 0.0358 and 0.0007, respectively. n ~ 5 animals, Unpaired t-Test)**.** Our findings demonstrate that K_{ATP} channels are predominantly expressed in 5HT neurons in the DRN, and their modulation influences feeding behavior, suggesting that these channels play an important role in food intake.

Disclosures: P.S.Z.B. Zarpellon Barbosa: None. **L.L.K. Elias:** None. **R.M. Leao:** None.

Poster

PSTR058: Other Ion Channels

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR058.21/C2

Topic: B.03. Ion Channels

Support: NIH Grant R01DC021671

Title: The role of Na^+leak channel NALCN in excitability and synaptic transmission of cerebellar unipolar brush cells

Authors: *C. CANDLER[1], K. E. WHITTAKER[2], T. S. BALMER[1];
[1]Sch. of Life Sci., [2]Arizona State Univ., Tempe, AZ

Abstract: NALCN is a Na^+ leak channel that is abundantly expressed in neurons and plays an important role in regulating spontaneous activity through its tonic inward leak current. NALCN has recently been shown to be modulated through GPCR activation by various neurotransmitters and neuromodulators. RNAseq data suggests high expression of NALCN channel complex genes in unipolar brush cells (UBCs), glutamatergic cerebellar interneurons that extend and transform mossy fiber signals to granule cells. UBCs can be classified into two subtypes based on their response to glutamate: ON UBCs which are predominately excited by glutamate through AMPA and mGluR1 receptors, and OFF UBCs which are inhibited by glutamate through mGluR2 activation. mGluR2 is a GPCR coupled to inhibitory G-proteins ($G_{i/o}$) which activate GIRK channels that produce an outward current. Here we address how NALCN functions in the two subtypes of UBCs to regulate spontaneous firing and whether they act as effectors that underly synaptic responses to glutamate. Whole-cell patch clamp recordings were made in sagittal

sections of P21-30 mouse cerebellum. NALCN current is increased in low extracellular Ca^{2+}. When extracellular Ca^{2+} was lowered from 1.5 mM to 0.1 mM a larger inward current was generated in OFF UBCs (102.30 pA ± 18.09 SEM, n = 6) than in ON UBCs (32.00 pA ± 1.74, SEM n = 4) (p = 0.0167), suggesting that NALCN may underlie different roles in the two subtypes. Brief glutamate puffs (1 mM 5-20 ms) onto OFF UBCs evoked an mGluR2-mediated outward current that was partially blocked by GIRK channel blocker Ba^{2+} and decreased significantly more during application of NALCN antagonist L-703606, showing that mGluR2-mediated modulation of NALCN may contribute to the outward current evoked by glutamate. In the presence of GIRK blockers, the mGluR2 agonist LY354640 generated an outward current (p = 0.0019, n=6), potentially due to the closure of the inward NALCN leak current. Taken together, these data support the conclusion that NALCN underlies a depolarizing tonic current in UBCs that regulates their spontaneous firing rate. Further experiments using conditional NALCN knockout mice will further explore the contribution of this inward leak to neuronal excitability and synaptic transmission.

Disclosures: C. Candler: None. **K.E. Whittaker:** None. **T.S. Balmer:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.01/C3

Topic: B.05. Synaptic Plasticity

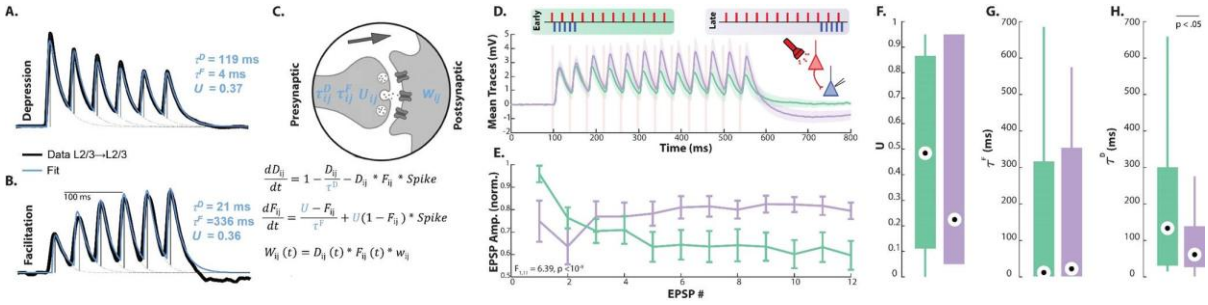
Support: NSF 2405476

Title: Synapses Learn to Tell Time

Authors: *J. MCDOWELL[1], D. V. BUONOMANO[1], S. SOLDADO-MAGRANER[2], S. ZHOU[2,3];
[1]Psychology/ Neurobio., UCLA, Los Angeles, CA; [2]Neurobio., Univ. of California, Los Angeles, Los Angeles, CA; [3]Computat. and Systems Neurosci., Fudan Univ., Shanghai, China

Abstract: One of the most fundamental tenets in neuroscience is that information is stored in the strength of synapses, and that learning relies on changes in synaptic strength. Here we aim to extend this tenet into the temporal domain by proposing that synapses not only learn to strengthen or weaken, but can learn *when* to be weak or strong on the subsecond scale. It has been known for more than half a century that synapses exhibit **short-term synaptic plasticity (STP)**: a form of presynaptic, use-dependent, plasticity in which consecutive spikes can elicit progressively depressing (short-term depression/paired-pulse depression) or facilitating (short-term facilitation/paired-pulse facilitation) postsynaptic potentials. Here we provide the first experimental evidence that STP may be learned. By pairing postsynaptic activity to co-occur with the first or last spikes of a presynaptic train, we shifted STP towards depression or facilitation, respectively. This result is consistent with our prediction that short-term synaptic

dynamics are tuned to peak at the time of postsynaptic activity. Finally, we show that when Learned-STP is incorporated into artificial neural network models, the STP parameters can be trained to generate interval selective neurons, solve the spatiotemporal XOR task, as well as a complex temporal Morse code recognition task. Together, these studies establish a novel experimental and theoretical framework to potentially extend our understanding of synaptic plasticity and synaptic computations into the temporal domain.**Fig. 1. A-B.** Short-term depression and facilitation at neocortical synapses and fits using the Tsodyks-Markram model. **C**. The Learned-STP hypothesis proposes that U, τ^F, and τ^D undergo experience-dependent learning. **D-E.** Early and late pairing induced differential STP. **D.** Average of the EPSPs recorded from ChR^+ neurons from the Early (green, n=12) and Late (purple, n=13) training groups. Shaded areas show the SEM. **E.** Quantification of actual EPSP amplitudes. There was a significant interaction between EPSP # and training group.



Disclosures: J. McDowell: None. **D.V. Buonomano:** None. **S. Soldado-Magraner:** None. **S. Zhou:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.02/C4

Topic: B.05. Synaptic Plasticity

Support: NIH/NINDS R01NS135048

Title: Synaptotagmin-3 and -7 mediate short-term facilitation via dynamic vesicle recruitment to the readily releasable pool

Authors: *D. WEINGARTEN, S. JACKMAN;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Short-term facilitation (STF) is a fundamental form of synaptic plasticity that enables neurons to dynamically modulate information transfer in response to patterns of presynaptic activity. Yet, the mechanisms underlying STF remain incompletely understood. Synaptotagmin-7

(SYT7) has been implicated as a key calcium sensor for STF, but residual facilitation persists in SYT7 knockout models, suggesting the involvement of additional molecular players. Here, we combined whole-cell electrophysiological recordings kinetic vesicle pool modeling to dissect the roles of synaptotagmin-3 (SYT3) and SYT7 in mediating STF at central synapses. Using single and double knockout (DKO) mice for both isoforms, we studied STF across the cerebellum, hippocampus, medial prefrontal cortex, and auditory brainstem. Strikingly, while loss of either SYT3 or SYT7 partially reduced STF, double knockout of both isoforms abolished all detectable facilitation at every synapse tested. To quantitatively analyze synaptic vesicle dynamics during STF, we developed a novel RRP (readily releasable pool) estimation algorithm, validated using both one- and two-pool vesicle models under facilitating and non-facilitating conditions. Our analysis revealed that STF reflects a rapid, reversible, frequency-dependent increase in the size of the RRP. In contrast, DKO synapses exhibited no such dynamic RRP expansion and lost all frequency dependence, implicating SYT3 and SYT7 as essential and synergistic regulators of RRP recruitment during repetitive activity. These findings establish dynamic regulation of the RRP as a core mechanism for activity-dependent plasticity and synaptic adaptation, orchestrated by coordinated actions of SYT3 and SYT7 on distinct time scales. Our validated modeling framework enables quantitative analysis of vesicle pool dynamics in diverse synapses, opening new avenues for probing the molecular logic of neural computation and its dysregulation in disease. Crucially, identifying SYT3 and SYT7 as key mediators of STF makes it possible to directly investigate the role of short-term facilitation in circuit function throughout the brain and to dissect its contributions to neuropsychiatric and neurological disorders.

Disclosures: D. Weingarten: None. **S. Jackman:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.03/C5

Topic: B.05. Synaptic Plasticity

Support: ANR (EUR Bordeaux graduate program and PREPLASH grant)
GPR BRAIN_2030 project (University of Bordeaux)

Title: Abrogation of presynaptic facilitation at hippocampal mossy fiber synapses impacts neural ensemble activity and spatial memory

Authors: *C. MARNEFFE[1], N. GROSJEAN[2], C. CHATRAS[2], A. MOREIRA DE SA[3], A. ERHARDT[2], S. LECOMTE[2], E. HARRELL[2], A. L. KEES[4], C. MULLE[5];
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Abstract: Presynaptic short-term plasticity is thought to play a major role in the process of spike transfer within local circuits. Mossy fiber synapses between the axons of dentate gyrus (DG) granule cells and CA3 pyramidal cells (Mf-CA3 synapses) display a remarkable extent of presynaptic plasticity which endows these synaptic connections with detonator properties. Here we have investigated the role of presynaptic facilitation at Mf-CA3 synapses in the operation of CA3 circuits in vivo and in memory encoding by analysing mice with selective abrogation of synaptotagmin 7 (Syt7) in DG granule cells (DG-Syt7 KO mice). We extend previous studies to show that short-term presynaptic facilitation is selectively suppressed at Mf-CA3 synapses in the absence of Syt7, without any impact on basal synaptic properties and on long-term potentiation. Short-term plasticity was found to be crucial for spike transfer between the DG and CA3 in conditions of naturalistic patterns of presynaptic firing. At the network level, in awake head-fixed mice, the abrogation of short-term plasticity largely reduced the co-activity of CA3 pyramidal cells. Finally, whereas DG-Syt7 KO mice show deficits in spatial memory tasks which rely on the process of pattern completion, and display altered emotional processes. These results give important insights into how the detonator properties of DG-CA3 synapses translate at the circuit and behavioral levels.

Disclosures: C. Marneffe: A. Employment/Salary (full or part-time): KULeuven. **N. Grosjean:** None. **C. Chatras:** None. **A. Moreira de Sa:** None. **A. Erhardt:** None. **S. Lecomte:** None. **E. Harrell:** None. **A.L. Kees:** None. **C. Mulle:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.04/C6

Topic: B.05. Synaptic Plasticity

Support: NR0011-25-3-0142

Title: Direct Identification of Spike-Timing Dependent Plasticity from Endogenous Spiking Activity During Learning

Authors: *C. SUN[1], B. S. ROBINSON[3], B. MOORE[1], X. ZHANG[1], D. SONG[1,2];
[1]Alfred E. Mann Dept. of Biomed. Engin., [2]Dept. of Neurolog. Surgery, USC, Los Angeles, CA;
[3]Applied Physics Lab., Johns Hopkins Univ., Laurel, MD

Abstract: Long-term synaptic plasticity (LTSP), driven by activity-dependent changes, is fundamental to neural information processing, learning, and memory. Spike-timing dependent plasticity (STDP), a Hebbian form of LTSP, plays a key role in theoretical models of learning, but its direct involvement during real-world behavior remains unclear due to the lack of methods to infer it from endogenous activity. To address this, we developed a computational framework that tracks time-varying functional connectivity and identifies STDP directly from spike train data recorded during learning.We applied this framework to simultaneous hippocampal

recordings from CA3 and CA1 neurons in rats performing a Delayed Nonmatch-to-Sample Learning (DNMSL) task. Our model estimates functional connectivity, dynamic synaptic weights, and underlying STDP rules using a subspace-based spiking neuron model. Parameters are optimized via mini-batch gradient descent to navigate complex loss surfaces and avoid local minima. Robustness was validated using synthetic datasets and Monte Carlo simulations to prevent overfitting.In experimental data spanning four DNMSL sessions, we first identified strongly connected neuron pairs. Within single sessions, the inferred STDP rules resembled canonical in vitro findings: long-term potentiation (LTP) occurred when presynaptic spikes preceded postsynaptic spikes (causal), while long-term depression (LTD) occurred for the reverse order (non-causal). Using concatenated spike data across consecutive sessions, we also identified a Hebbian rule where repeated co-activation strengthened synaptic connections over time.These results demonstrate that STDP operates during behavioral learning and can be inferred directly from endogenous activity using our model. This work provides a powerful tool for studying how time-varying synaptic plasticity mechanisms contribute to memory formation in vivo.

Disclosures: C. Sun: None. **B.S. Robinson:** None. **B. Moore:** None. **X. Zhang:** None. **D. Song:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.05/C7

Topic: B.05. Synaptic Plasticity

Support: EMSTAR/22/078

Title: Short-term plasticity at DG-CA3 synapses enables phase precession, pattern separation, and place cell remapping

Authors: *N. SINGH[1], S. NADKARNI[2];
[1]Biol., Indian Inst. of Sci. Educ. and Research, Pune, Pune, India; [2]Biol., IISER Pune, Pune, India

Abstract: The autoassociative network of hippocampal CA3 pyramidal neurons (CA3p) is posited to encode and retrieve episodic memories. Effective memory storage in such a recurrent network requires decorrelated or orthogonalized input patterns. Experimental studies have implicated the dentate gyrus (DG) in performing this pattern separation, although the specific cellular and synaptic mechanisms remain poorly understood.
A critical feature of the DG-CA3 pathway is its unique synaptic transmission. Granule cells of the DG send axons via mossy fibers (MF) to CA3p. These synapses exhibit strong short-term facilitation while also recruiting feedforward inhibition via interneurons. This synaptic arrangement suggests a dynamic interplay of excitation, inhibition, and plasticity that contributes

to temporal and spatial coding in CA3.
We developed a biophysical computational model comprising a network of three components: MF bouton (MFB), a HH-type model of CA3p, and fast-spiking inhibitory interneurons. The MFB is a phenomenological model based on short-term plasticity dynamics from a detailed synaptic model, where presynaptic morphology underlies transient neurotransmitter modulation. Excitatory inputs from the MF synapses target both CA3p and interneurons, the latter providing GABA-mediated feedforward inhibition to CA3p.
Using this model, we demonstrate that STP at DG-CA3 synapses is critical for generating phase precession in CA3p. Facilitation of theta-modulated DG input progressively shifts CA3 firing phase across theta cycles, even in the absence of recurrent collaterals. Furthermore, we show that STP is essential for achieving pattern separation in CA3. In models lacking STP, CA3 output patterns exhibit significantly reduced temporal and spatial decorrelation. This effect arises from synaptic "detonation," where the facilitated MF input overcomes inhibition and abruptly triggers postsynaptic action potentials.
Interestingly, while DG granule cells exhibit highly selective place fields, downstream CA3 representations are broader, consistent with in vivo observations. This transformation is driven by the dynamics of STP and inhibition. Finally, we show that variability in DG-CA3 synaptic strength can induce place cell remapping in CA3 neurons in response to changes in environmental cues.
Together, these results highlight that STP at DG-CA3 synapses is not merely a modulatory feature but a core computational mechanism enabling phase coding, pattern separation, and flexible spatial representations in the hippocampus.

Disclosures: N. Singh: None. **S. Nadkarni:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.06/C8

Topic: B.05. Synaptic Plasticity

Support: KAKENHI Grant JP23H05476

Title: Assembly-specific inhibition improves replay diversity in spiking recurrent networks

Authors: *T. BARTA;
Neural Coding and Brain Computing unit, OIST, Okinawa, Japan, Okinawa, Japan

Abstract: The brain is required to store a large number of memories. The limits on the number of memories that can be reliably stored in a neural network - the memory capacity - has been theoretically extensively studied. However, such studies typically study persistent neural activity, which is not always biologically plausible. In contrast, spontaneous transient activation of memories during offline activity is essential for memory stabilization and consolidation. Here we

propose a biologically motivated definition of a network's memory capacity: We assert that a network needs to be capable of spontaneously replaying a pattern for the memory to be reliably stored and propose the number of memories available for spontaneous replay as a measure of the network's memory capacity. We simulate spiking networks of adapting leaky integrate-and-fire neurons with large number of hard-wired sparse memory assemblies and stabilize the network by allowing plasticity of inhibitory-to-excitatory synapses (I to E). We used three different plasticity rules leading to different levels of I to E connectivity structure complexity. The most complex rule, forming weak inhibitory assemblies mirroring the excitatory assemblies, lead to the most diverse replay activity, substantially increasing the number of retrievable memories. Our results highlight the importance of stimulus-specific inhibitory structure and suggest that inhibitory neurons play an active role in memory storage beyond mere network stabilization.

Disclosures: T. Barta: None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.07/C9

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

Support: 2016/IF60611
EMSTAR/2023/SL04

Title: Acetylcholine determines the dynamics of pattern formation and pattern completion in CA3 recurrent networks

Authors: *R. SHARMA[1], S. NADKARNI[2];
[1]IISER PUNE, Pune, India; [2]IISER Pune, Pune, India

Abstract: Neuromodulation by acetylcholine is critical for memory formation and retrieval. The hippocampus, the locus of episodic memory formation, integrates spatiotemporal cues with multidimensional contextual information to synthesize episodic memories in autoassociative CA3 networks. Acting through its various receptors, acetylcholine modulates synaptic efficacy and neuronal excitability. Activation of M4 receptors reduces neurotransmitter release by suppressing calcium influx from voltage-gated calcium channels. At the same time, M1 receptor activation enhances neuronal excitability by suppressing hyperpolarizing potassium channels. We constructed a spiking network using simple neuron models of excitatory and inhibitory populations. Excitatory synapses generate short-term synaptic plasticity (STP) dynamics that exhibit both short-term facilitation and depression. In contrast, inhibitory neurons have purely depressing synapses. We implemented synaptic connectivity based on a Hebbian plasticity rule, which implies stronger connections between neurons with correlated activity, as observed in these networks. Acetylcholine acts by suppressing neurotransmitter release in excitatory synapses and increasing neuronal excitability due to a reduced hyperpolarizing adaptation

current. Our simulations demonstrate how neuromodulation by acetylcholine enables the retrieval of complete activity patterns from partial or incomplete stimuli. The action of acetylcholine enhances the retrieval of embedded patterns because the altered STP filters out low-frequency noise, and increased excitability amplifies recurrent excitation of embedded patterns only for strongly correlated stimuli. With low acetylcholine, the network shows the natural emergence of replay-like events observed during sharp-wave ripple (SWR) events during periods of slow-wave sleep (SWS). We also explore the role of theta-frequency modulation in these networks and its interactions with synaptic dynamics and membrane time constants. Our simulations suggest that the differential expression of acetylcholine receptors enables complex signal processing mechanisms across the network that facilitate efficient encoding and retrieval of episodic memories.

Disclosures: R. Sharma: None. **S. Nadkarni:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.08/C10

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS091546

Title: Non-ionic signaling rapidly remodels postsynaptic DLG to induce retrogradehomeostatic plasticity

Authors: *C. QIU[1,2], S. PERRY[3], Y. HAN[2], J. ZHUANG[3], P. GOEL[3], D. K. DICKMAN[3]; [2]Neurosci. Grad. Program, [3]Neurobio., [1]USC, Los Angeles, CA

Abstract: Synapses must be resilient to the challenges they confront during development, experience, disease, and aging. Loss of synaptic stability is associated with a variety of neurological and neurodegenerative diseases. At the glutamatergic *Drosophila* neuromuscular junction (NMJ), acute pharmacological blockade of postsynaptic glutamate receptors (GluRs) is counteracted by enhanced presynaptic neurotransmitter release to maintain synaptic strength. Such retrograde adaptive plasticity is termed presynaptic homeostatic potentiation (PHP). Much is now known about the genes and mechanisms in the presynaptic neuron that enable the expression of PHP. However, how retrograde PHP signaling is rapidly induced in the postsynaptic compartment remains enigmatic. One clue has recently emerged, suggesting that perturbation of ionic flow through GluRs is insufficient to induce rapid PHP expression. Here, we use molecular genetics, electrophysiology, pharmacology, and confocal and super-resolution microscopy to demonstrate that rapid PHP induction utilizes conformational GluR signaling to remodel the postsynaptic apparatus. First, pharmacological antagonism of GluRs provokes changes in nanoscale receptor organization, which is propagated throughout the postsynaptic compartment. These structural postsynaptic changes are necessary for the presynaptic structural

remodeling that characterizes PHP, including enhanced active zone size and intensity. Second, using a CRISPR-based genetic screen, we identify Discs large (Dlg), the fly homolog of mammalian PSD-95, as a key postsynaptic scaffold targeted for conformational signaling selectively required for rapid PHP expression. Finally, we interrogated whether synaptic activity itself was necessary for PHP inductive signaling. Surprisingly, we find that homeostatic remodeling of both pre- and post-synaptic compartments persists following pharmacological diminishment of GluRs in the absence of neurotransmission. Together, our findings support a model in which acute perturbation of GluRs triggers an activity-independent conformational signaling that is propagated throughout the postsynaptic apparatus, transmitting retrograde information to induce presynaptic homeostatic potentiation.

Disclosures: C. Qiu: None. **S. Perry:** None. **Y. Han:** None. **J. Zhuang:** None. **P. Goel:** None. **D.K. Dickman:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.09/C11

Topic: B.05. Synaptic Plasticity

Support: NSF GRFP

Title: Rim selectively promotes transmission and plasticity at high release synapses

Authors: *R. C. STARK, D. K. DICKMAN;
Neurobio., USC, Los Angeles, CA

Abstract: Rab3-interacting molecules (RIMs) are highly conserved, core active zone (AZ) proteins that promote synaptic vesicle release. In *Drosophila*, a single gene encodes *rim*, and prior research at the larval neuromuscular junction (NMJ) has shown that Rim localizes presynaptic voltage-gated calcium channels, promotes release probability, and regulates the size of the readily releasable vesicle pool (RRP). Studies in both mammals and flies have shown that loss of *rim* severely reduces neurotransmission. To determine whether Rim is universally required or functions in a synapse-specific manner, we studied its role at two distinct fly motor neuron inputs: strong, phasic “Is” synapses and weak, tonic “Ib” synapses. Using selective botulinum toxin expression to isolate each input electrophysiologically, we unexpectedly found that *rim* is essential for evoked release at Is synapses but dispensable at Ib synapses. Indeed, a series of electrophysiological assays and imaging studies all show that Rim is crucial for determining several aspects of Is synapses’ characteristics (relatively high release probability, tight calcium channel-vesicle coupling distances, and large RRP size) and essentially dispensable for these aspects of Ib inputs. Interestingly, this synapse type-specific specialization extends to homeostatic plasticity: Rim is required for presynaptic potentiation at Is terminals following pharmacological postsynaptic receptor blockade but is not necessary for chronic homeostatic

plasticity at Ib terminals. Together, our findings indicate that a core AZ scaffold, Rim, selectively functions at diverse synaptic subtypes to promote neurotransmission and enable adaptive plasticity.

Disclosures: R.C. Stark: None. **D.K. Dickman:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.10/C12

Topic: B.05. Synaptic Plasticity

Support: DFG award DU331/6-3

Title: Calcium mediated presynaptic homeostatic plasticity at the Drosophila NMJ

Authors: *L. DENEKE[1], J. ARIAN[1], N. KRICK[1], U. THOMAS[2], C. DUCH[1];
[1]Inst. of Developmental and Neurosci., Johannes Gutenberg Univ. of Mainz, Mainz, Germany;
[2]Leibniz Insitute For Neurobio., Magdeburg, Germany

Abstract: At the presynaptic terminal of chemical synapses numerous calcium-dependent processes like synaptic vesicle (SV) release, SV recycling, and synaptic plasticity occur side by side and must be coordinated. At the *Drosophila* neuromuscular junction (NMJ), an established model for basic functions of glutamatergic synapses, we previously found separate regulation of calcium-dependent SV release and recycling through the division of labor between two voltage-gated calcium channels (VGCCs). The Cav2 homolog cacophony (cac) is required for evoked SV release at active zones, while the Cav1 homolog DmCa1D localized outside active zones to augment SV recycling and short-term plasticity. The calcium extrusion pump PMCA isolates these functions from each other (Krick et al., PNAS, 2021). We now report that both, PMCA and DmCa1D are also required for the initiation of presynaptic homeostatic plasticity (PHP). At the *Drosophila* NMJ, PHP is regulated by numerous mechanisms that adjust release probability (Pr). Moreover, PHP is divided into genetic and mechanistically separable initiation and maintenance phases. During PHP initiation Pr is increased by an upregulation of the number of cac VGCCs in the active zone (Ghelani et al., Science Advances, 2023). Combining Drosophila genetics, electrophysiology and imaging techniques we now test whether signaling downstream of Cav1 and PMCA is required for PHP maintenance. A key open question is whether synapses regain the capacity to re-initiate PHP in response to second and third hit perturbations. This would require resetting the number of active zone cac channels and maintaining increased Pr by alternative mechanisms, thus enabling PHP re-initiation though additional cac insertion into active zones. As candidate mechanisms for retaining increased Pr during PHP maintenance we focus on the role of ER calcium-signaling and the regulation of plasma membrane (PM) ER interactions. First data indicate that the tuning of store operated calcium entry (SOCE) via STIM-Orai channels and/or calcium induced calcium release (CICR) through ryanodine receptors at newly formed

PM-ER contacts may be key in keeping Pr up during PHP maintenance while restoring the adaptive potential of PMCA and Cav1 to scale up Pr.

Disclosures: L. Deneke: None. **J. Arian:** None. **N. Krick:** None. **U. Thomas:** None. **C. Duch:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.11/C13

Topic: B.05. Synaptic Plasticity

Support: NIH Grant GR1066085

Title: A genetic screen identifies the glutamate receptor subunit Ekar to be required for presynaptic homeostatic plasticity

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Abstract: Synaptic transmission faces constant challenges throughout an organism's lifespan, necessitating robust homeostatic mechanisms. While these processes stabilize neural activity, the underlying molecular machinery remains largely unexplored. Our lab recently identified the presynaptic glutamate receptor (GluR) subunit KaiRID and its auxiliary subunit dSol-1 as crucial presynaptic autoreceptors for both basal transmission and presynaptic homeostatic potentiation (PHP). To systematically identify additional GluR subunits involved in these vital processes, we conducted a comprehensive study of all 16 GluRs encoded in the Drosophila genome. Representing all major ionotropic and metabotropic GluR classes (Kainate, AMPA, NMDA, Glutamate-gated Chloride, and metabotropic), we first mapped the expression of these receptors at the larval neuromuscular junction (NMJ) and in the adult brain. We found diverse expression patterns, with 9 out of 16 GluRs expressed in motor neurons, 5 exclusively in postsynaptic muscle, and 2 apparently absent during larval stages. In the adult brain, all 16 GluRs were expressed in various regions, except for the 5 muscle-specific subtypes. Next, leveraging CRISPR/Cas9, we generated null mutants for all 16 GluRs and screened the 9 motor neuron-expressed receptors for roles in NMJ development and function. Surprisingly, loss of any single motor neuron-expressed GluR did not significantly impact synaptic growth or baseline neurotransmission. However, our investigation revealed a specific requirement for the eye-enriched kainate receptor subunit (Ekar) in the *chronic* expression of presynaptic homeostatic potentiation. Our ongoing research is focused on elucidating the potential interaction between Ekar, KaiRID, and dSol-1, and the precise mechanisms by which Ekar selectively promotes the sustained enhancement of presynaptic release during homeostatic plasticity. This genome-wide analysis provides a detailed atlas of GluR expression and function at the synapse, revealing a specialized role for the kainate receptor Ekar in a critical form of long-term synaptic plasticity.

Disclosures: J.C. Martinez: None. **C. Chien:** None. **N.L. Tran:** None. **D.K. Dickman:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.12/C14

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS091546
NIH Grant NS126654

Title: Distinct input-specific mechanisms enable presynaptic homeostatic plasticity

Authors: C. CHIEN[1], K. HE[2], S. PERRY[3], E. TCHITCHKAN[2], Y. HAN[4], X. LI[2], ***D. K. DICKMAN**[2];
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Abstract: Synapses are endowed with the flexibility to change through experience, but must be sufficiently stable to last a lifetime. This tension is illustrated at the *Drosophila* neuromuscular junction (NMJ), where two motor inputs that differ in structural and functional properties co-innervate most muscles to coordinate locomotion. To stabilize NMJ activity, motor neurons augment neurotransmitter release following diminished postsynaptic glutamate receptor functionality, termed presynaptic homeostatic potentiation (PHP). How these distinct inputs contribute to PHP plasticity remains enigmatic. We have used a botulinum neurotoxin to selectively silence each input and resolve their roles in PHP, demonstrating that PHP is input-specific: Chronic (genetic) PHP selectively targets the tonic MN-Ib, where active zone remodeling enhances Ca^{2+} influx to promote increased transmitter release. In contrast, acute (pharmacological) PHP selectively increases vesicle pools to potentiate phasic MN-Is. Thus, distinct homeostatic modulations in active zone nanoarchitecture, vesicle pools, and Ca^{2+} influx collaborate to enable input-specific PHP expression.

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Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.13/C15

Topic: B.05. Synaptic Plasticity

Support: Grinnell College

Title: Computational Simulation of Synaptic Cleft pH and the Role of Protons in Presynaptic Homeostatic Potentiation at the Vertebrate Neuromuscular Junction

Authors: *M. NODA[1], E. ALBRECHT[1], B. KAYNAK[2], T. BARTOL[2], T. J. SEJNOWSKI[2], C. A. LINDGREN[1];
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Abstract: The neuromuscular junction (NMJ) transmits acetylcholine (ACh) from motor neuron terminals to the end-plates on muscle fibers. Any compromise in post-synaptic ACh receptor function is compensated by an increase in ACh release, a phenomenon known as presynaptic homeostatic potentiation (PHP). We have shown that pharmacological blocking of the acid-sensing ion channels (ASICs) at the NMJ prevents PHP. Since ASICs are cation channels that are normally activated by protons, we hypothesize that a decrease in extracellular pH in the synaptic cleft mediates the increase in quantal ACh release during PHP. This has been supported by the observation that acidification of the extracellular space using pH-controlled buffers increases ACh release, an effect that depends on ASICs and occludes PHP. Microscopic images of the NMJs of mice infected with the AAV virus containing pHluorin (pH-sensitive probe) show potential for measuring the cleft's pH. These studies support the theory that partial nAChR blockage leads to synaptic cleft acidification, activating the ASIC channels on the presynaptic nerve terminal. However, experimental measurement of the intracellular pH of the NMJ requires precise techniques and does not allow for flexible manipulation of chemical variables, such as blockage of specific channels, and how they impact the overall pH and thus the induction of the PHP. A non-stochastic simulation model at Drosophila NMJ by Feghhi and colleagues has shown a brief cleft acidification followed by alkalinization due to the pumping of the protons by the plasma membrane calcium-ATPase (PMCA), but questions about the impact of stochastic neurotransmitter release on the cleft pH remain. To address these challenges, we applied computational simulations using MCell/CellBlender packages to run Monte Carlo simulations of stochastic ACh release at the mouse NMJ. To simulate and track the pH of the cleft, we included chemical reactions, diffusion kinetics, and coupling effects between reactions that can impact the pH. The model allows manual adjustment of variables such as ion concentrations and kinetics, allowing us to specify the most important variables and cellular processes that result in retrograde signaling following cleft acidification.

Disclosures: M. Noda: None. **E. Albrecht:** None. **B. Kaynak:** None. **T. Bartol:** None. **T.J. Sejnowski:** None. **C.A. Lindgren:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.14/C16

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R35NS097212
NIH Grant R35NS137247
Simons Foundation
Life Sciences Research Foundation (LSRF)
Simons SFARI
NRF of Korea 800-20240519

Title: Synaptic Semaphorin Signaling Drives Presynaptic Homeostatic Plasticity in the Adult Mammalian Brain

Authors: *U. LEE, P. H. CHIPMAN, B. O. ORR, R. D. FETTER, G. W. DAVIS;
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Abstract: Semaphorins, as well as their receptors, co-receptors, and immediate downstream signaling proteins, have been assigned as high-confidence risk factors for autism, intellectual disability, and epilepsy. A prevailing perspective is that altered axon guidance, caused by defective Semaphorin signaling, leads to altered circuit construction with adverse mental health outcomes. Certainly, this cannot be ignored. Yet, there is increasing evidence to support a synaptic origin for many disorders, including multiple neurodevelopmental, neuropsychiatric, and neurodegenerative diseases. Presynaptic homeostatic plasticity (PHP) is a potent form of adaptive plasticity that has been documented at synapses as diverse as the glutamatergic *Drosophila* neuromuscular junction (NMJ), cholinergic mammalian NMJ (including human), and glutamatergic synapses in the mammalian brain. Although work in the neuromuscular systems has suggested molecular links between PHP and neurological disorders, this remains unexplored within the mammalian brain. We recently demonstrated that a secreted class III Semaphorin (Sema3a) is a unifying, trans-synaptic signal, necessary for PHP at highly divergent synapses. Sema3a acts acutely at the cholinergic mouse NMJ and synapses in the adult hippocampus (*in vitro* and *in vivo*) to drive expression of PHP. Experimental evidence includes slice electrophysiology, direct optical analysis of excitatory transmission, three-dimensional electron microscopy (3D EM) reconstruction of synaptic circuitry, and animal behavior. Mechanistically, Sema3a promotes vesicle redistribution from a non-releasing to recycling and readily releasable vesicle pools. We are actively engaged in determining the presynaptic Sema3a receptor complex that is required for PHP. To date, this signaling complex includes PlexinA4 as well as Beta-Integrin, acting as a conserved co-receptor. Conservation of downstream signaling will be presented, including putative conserved roles for Focal Adhesion Kinase (FAK) and presynaptic Talin. A stated goal is to determine how the engagement of the presynaptic Sema3a receptor complex re-partitions synaptic vesicle pools, thereby enabling expression of PHP. Collectively, this widespread utilization of common PHP mechanisms emphasizes the translational potential of model organisms toward promoting neuronal resilience to combat brain disorders and disease.

Disclosures: U. Lee: None. **P.H. Chipman:** None. **B.O. Orr:** None. **R.D. Fetter:** None. **G.W. Davis:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.15/C17

Topic: B.05. Synaptic Plasticity

Support: NIH Grant Number R35NS137247 to GWD

Title: The spatial scale of presynaptic homeostatic plasticity in a hippocampal microcircuit

Authors: *P. H. CHIPMAN, R. FETTER, G. W. DAVIS;
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Abstract: Homeostatic synaptic plasticity is thought to buffer against neural circuit dysfunction via alterations to the strength of synapses. Presynaptic homeostatic plasticity (PHP) is a powerful form of homeostatic plasticity that is engaged following postsynaptic perturbations to precisely recover the gain of synaptic transmission primarily via alterations in presynaptic neurotransmitter release. We have recently demonstrated that PHP is engaged at pyramidal cell synapses in the adult mouse hippocampus in response to partial antagonism of AMPA receptors. Structurally, PHP is expressed via coordinated ultrastructural expansion of trans-synaptic components including active zones, spine volumes, and the number of docked synaptic vesicles (collectively referred to as structural PHP or sPHP). Whether other cell types in the hippocampal microcircuit, including local inhibitory neurons, also exhibit sPHP remains unknown. Using cell-type specific labeling of inhibitory cell types compatible for volume electron microscopy we demonstrate cell-type specific expression of sPHP in the mouse hippocampus *stratum oriens*. This cell-type specific plasticity is Sema3a-dependent and results in spatial gradient of synaptic morphologies in a distance-dependent manner surrounding inhibitory cell dendrites. Our data suggest that secreted homeostatic synaptotropic cues generate a topography of synaptic strengths across the local microcircuitry to achieve synaptic homeostasis.

Disclosures: P.H. Chipman: None. **R. Fetter:** None. **G.W. Davis:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.16/C18

Topic: B.05. Synaptic Plasticity

Support: NIG Grant NS091546

Title: Extreme loss of postsynaptic glutamate receptors reveals the limits of presynaptic homeostatic compensation

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[1]Dept. of Neurobio., [2]Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: Robust information transfer in the nervous system relies on homeostatic processes at synapses that stabilize activity. At the glutamatergic Drosophila neuromuscular junction (NMJ), previous studies have established that loss of postsynaptic glutamate receptors (GluRs) triggers presynaptic homeostatic potentiation (PHP), an adaptive retrograde signaling system where enhanced presynaptic neurotransmitter release precisely maintains synaptic strength. However, although previous studies have suggested presynaptic glutamate release robustly and reliably scales with GluR loss, the limits of PHP compensation have not been directly tested. Here, we systematically screened various genetic manipulations to find a condition where >90% of GluRs are eliminated. Preliminary data suggest that in the most critical condition we have characterized, synaptic strength failed to be fully restored to baseline level when miniature EPSP is drastically reduced to nearly undetectable levels, indicating no apparent increase in quantal content as is normally observed. These findings suggest that PHP might have reached a ceiling and cannot fully counterbalance severe postsynaptic deficits. Ongoing experiments are focused on using independent electrophysiological measurements to quantify presynaptic release and developing immunostaining assays to characterize the pre- and post-synaptic structures under near-complete GluR loss. Our data support a model where PHP plasticity is limited that only limited postsynaptic receptor loss can be fully counteracted. This ceiling effect places important constraints on how nervous systems maintain stability in the face of myriad perturbations that occur during development, maturation, experience, aging, and disease.

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Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR059.17/C19

Topic: B.05. Synaptic Plasticity

Support: University Research Priority Program ‘Adaptive Brain Circuits in Development and Learning’ grant by the University of Zurich to MB and MM

Title: Distinct Presynaptic Homeostatic Adaptations to Activity Perturbations in Human Synapses

Authors: *M. BRASILI[1,2], K. SCHMIDT[1,2], B. WATMUFF[3], B. KAGAN[3], M. MUELLER[1,2];
[2]Dept. of Mol. Life Sci., [1]Univ. of Zurich, Zurich, Switzerland; [3]Cortical Labs, Melbourne, Australia

Abstract: Homeostatic plasticity maintains neuronal activity within adaptive boundaries. Most studies have focused on synaptic scaling, a postsynaptic form of homeostatic plasticity that involves the regulation of neurotransmitter receptors. Synaptic scaling stabilizes synaptic transmission across systems, including human iPSC-derived neuronal cultures. While presynaptic forms of homeostatic plasticity have been observed in diverse systems, they have not been reported for human synapses. Here, we investigated homeostatic plasticity after two neuronal activity perturbations in human iPSC-derived excitatory cortical i3 neurons after NGN2 induction co-cultured with human astrocytes. Chronic action potential (AP) blockade induced by TTX treatment (24h) increased the amplitude of synaptic miniature EPSCs (mEPSCs), suggesting synaptic up-scaling, consistent with previous work. Intriguingly, we also noted a marked increase in mEPSC frequency, indicative of presynaptic compensation. In line with presynaptic modulation, confocal microscopy revealed increased anti-Synapsin 1 fluorescence intensity after TTX treatment. Next, we examined synaptic transmission after neurotransmitter receptor impairment using the AMPA receptor antagonist Gyki-53655. Acute Gyki application at a sub-saturating concentration reduced mEPSC and AP-evoked EPSC (eEPSC) amplitude. After Gyki incubation for 24h, mEPSC amplitudes remained reduced, whereas eEPSC amplitudes returned towards baseline levels, implying presynaptic compensation. Furthermore, paired-pulse ratios were decreased after Gyki treatment for 24h, suggesting increased release probability (p_r). AP train stimulation revealed increased short-term depression without major changes in cumulative quantal content, consistent with increased p_r without major changes in readily-releasable vesicle pool size. In contrast to TTX, anti-Synapsin 1 fluorescence intensity was unchanged after Gyki application, indicating distinct presynaptic mechanisms. To assess neuronal network adaptations, we performed multi-electrode array recordings. First, no marked changes in mean firing rate were observed between Gyki-treated and untreated cultures between 1h and 24h. Gyki washout after 24h induced a 4-fold increase in mean firing rate, which gradually declined over 3h and returned to baseline after 24h, indicating a compensatory adjustment of network activity in response to chronic receptor perturbation. Together, our findings demonstrate distinct presynaptic homeostatic adaptations after two neuronal activity perturbations in human synapses.

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Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.18/Web Only

Topic: B.05. Synaptic Plasticity

Support: DGAPA-PAPIIT IN202825
SECIHTI-CVU-928160

Title: Bidirectional modulation of synaptic plasticity by memory extinction in the taste aversion circuit

Authors: *C. TADEO[1], B. GUTIERREZ[2], M. L. ESCOBAR[3];
[1]UNAM, Mexico city, Mexico; [2]Neurodesarrollo y Fisiología, Inst. de Fisiología Celular, Ciudad de México, Mexico; [3]UNAM, Fac Psicologia, Mexico City, Mexico

Abstract: The formation and extinction of memories are governed by dynamic modifications in synaptic plasticity, regulated by homeostatic mechanisms. Among these, metaplasticity plays a critical role by adjusting synaptic plasticity thresholds in response to prior neuronal activity. Our laboratory has previously demonstrated that conditioned taste aversion (CTA) training prevents the induction of both long-term potentiation (LTP) and long-term depression (LTD) in the basolateral amygdala to insular cortex (BLA-IC) pathway *in vivo*. However, how memory extinction affects subsequent synaptic plasticity, particularly LTD, remains poorly understood. Here, we show that CTA extinction partially restores the capacity for LTD expression in the BLA-IC pathway. Furthermore, quantitative analysis reveals a significant reduction in brain-derived neurotrophic factor (BDNF) levels in the insular cortex following extinction—a change consistent with metaplastic regulation—compared to subjects that retained the aversive memory. These findings demonstrate that CTA extinction bidirectionally modulates synaptic plasticity in the BLA-IC network, partially reversing the LTD suppression induced by aversive learning. Notably, this restoration of synaptic plasticity is accompanied by decreased BDNF expression in the insular cortex after extinction.

Disclosures: C. Tadeo: None. **B. Gutierrez:** None. **M.L. Escobar:** None.

Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.19/C20

Topic: B.05. Synaptic Plasticity

Support: NIH Grant EY025613

Title: Prey capture learning drives critical period-specific plasticity in mouse binocular visual cortex

Authors: *D. BISSEN[1], B. A. CARY[1], A. ZHANG[1], K. A. SAILOR[2], S. D. VAN HOOSER[1], G. G. TURRIGIANO[1];
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Abstract: Critical periods are developmental windows of high experience-dependent plasticity essential for the correct refinement of neuronal circuitry and function. While the consequences for the visual system of sensory deprivation during the critical period have been well-characterized, far less is known about the effects of enhancing sensory experience, for instance

by learning. Here, we use prey capture learning to assess structural and functional plasticity mediating vision-dependent learning in the primary visual cortex of critical period mice.We show that prey capture learning improves temporal frequency discrimination and drives a profound remodeling of visual circuitry through an increase in excitatory connectivity and spine turnover. This global and persistent rewiring is not observed in adult hunters and mediated by TNFα-dependent mechanisms. Our findings demonstrate that learning in a naturalistic paradigm during the critical period can drive structural plasticity and improve visual function, and promotes a long-lasting increase in spine dynamics that could enhance subsequent plasticity.

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Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR059.20/C21

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R01EY025613

Title: Rapid prey capture learning resets V1 activity set points in vivo

Authors: *D. P. LEMAN[1], B. A. CARY[2], D. BISSEN[2], B. J. LANE[2], N. F. WONG[2], R. SHANLEY[2], K. BHUT[2], G. TURRIGIANO[2];
[2]Biol., [1]Brandeis Univ., Waltham, MA

Abstract: Neocortical neurons possess stable firing rate set points to which they faithfully return when perturbed. These activity set points are established early in development and are stable through adulthood, suggesting they are an immutable feature of cortical networks. Here we challenge this idea using an ethological vision-dependent prey capture learning paradigm in juvenile rats. We employed a combination of behavioral analysis, chemogenetics, in vivo electrophysiology, spine imaging, and pharmacology to probe how naturalistic learning affects V1 activity. We find that rapid prey capture learning required visual cortex (V1) and enhanced the tuning of V1 neurons to specific behavioral epochs across learning sessions. Chronic electrophysiology recordings revealed a slow, state-dependent increase in V1b firing rates that began after learning was complete and persisted for days. This upward firing rate plasticity was gradual, gated by wake states, and in L2/3 neurons, was accompanied by a TNFα-dependent increase in excitatory synapses onto pyramidal neurons - all features of homeostatic plasticity within V1. Finally, blocking TNFα signaling after learning reduced the retention of hunting skills. Therefore, our findings demonstrate the malleability of firing rate setpoints in the visual critical period and present a role for homeostatic mechanisms in stabilizing the retention of learned visuomotor skills. Our results here illuminate the dynamic interplay of plasticity mechanisms in cortical networks that occurs during naturalistic behaviors.

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Poster

PSTR059: Synaptic Plasticity: Short-Term Dynamics and Homeostatic Adaptation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR059.21/C22

Topic: B.05. Synaptic Plasticity

Support: Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica, UNAM (PAPIIT) IN202825
Consejo Nacional de Ciencia y Tecnología (CONACYT) CVU-998761

Title: Acute exposure to enriched environment attenuates in vivo insular cortex LTD

Authors: *B. GUTIERREZ[1], C. TADEO[2], M. L. ESCOBAR[3];
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[3]UNAM, Fac Psicologia, Mexico City, Mexico

Abstract: Environmental enrichment (EE) has been widely recognized for its ability to enhance learning and memory through modulation of synaptic plasticity. Learning and memory expression rely on synaptic plasticity processes, which in turn are regulated by mechanisms such as metaplasticity, where neurons modulate their synaptic strength depending on their previous history of activity. The role of long-term depression (LTD) in cognitive processes, particularly those involving neocortical circuits, remains poorly explored. Our previous work demonstrated that a brief exposure to an EE affects the long-term potentiation (LTP) maintenance in the basolateral nucleus of the amygdala (BLA) to the insular cortex (IC) pathway, which is essential for the formation of aversive memories. The present study aimed to analyze whether EE exposure modifies the expression of in vivo LTD in the BLA-IC projection. To do so, adult male Wistar rats were exposed for seven days to an EE before LTD induction in the Bla-IC pathway. Our results show that the magnitude of LTD was clearly attenuated in enriched animals compared to controls. These findings show that EE modulates the expression of LTD in a neocortical area that constitutes a multisensory information node, widely related to the processing of aversive memories

Disclosures: B. Gutierrez: None. **C. Tadeo:** None. **M.L. Escobar:** None.

Poster

PSTR060: Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR060.01/C23

Topic: B.07. Network Interactions

Title: Comparative Performance of Neural-Specific Media in Differentiating and Maturing Human Neural Progenitor Cell-Derived Forebrain Neurons

Authors: G. YOGARAJAH[1], L. CHEW[1], S. A. LOUIS[2], A. EAVES[2,3], ***E. KNOCK**[4,5];
[1]STEMCELL Technologies, Vancouver, BC, Canada; [2]STEMCELL Technologies Inc, Vancouver, BC, Canada; [3]Terry Fox Lab., BC Cancer, Vancouver, BC, Canada; [4]STEMCELL Technologies Inc., Vancouver, BC, Canada; [5]Biol., Simon Fraser Univ., Burnaby, BC, Canada

Abstract: The choice of neural-specific culture medium is critical for directing neural progenitor cells (NPCs) through key developmental transitions toward mature neuronal phenotypes. Optimization of medium components ensures reproducibility for applications such as disease modeling and drug screening. In this study, we compared the performance of BrainPhys™ Neuronal Medium (with recommended supplements) and STEMdiff™ Forebrain Neuron Kit against non-validated media in supporting the differentiation and maturation of cryopreserved NPCs into forebrain neurons. Differentiation was performed using the SCTi003-A human induced pluripotent stem cell line-derived NPCs, seeded at 50,000 cells/cm² on poly-L-ornithine/laminin-coated surfaces. Cultures were then transitioned to either STEMdiff™ Forebrain Neuron Maturation Medium, BrainPhys™ Neuronal Medium with supplements, or published media formulation (Qi et al. Nat. Biotech. 2017) for an additional two weeks of maturation. Cultures maintained in non-validated media exhibited a ~60% reduction in neuronal confluence compared to those matured with STEMdiff™ or BrainPhys™ media (31.3 ± 4.1%, 97.1 ± 1.5%, and 99.2 ± 0.5% , respectively; mean ± SEM; n = 5 experiments). These morphological differences were corroborated by reduced expression of inhibitory neuron marker GABA in the unqualified media condition with STEMdiff™ or BrainPhys™ media (8.4 ± 3.8%, 43.4 ± 7.9% and 58.0 ± 17.7%, respectively; mean ± SEM; n = 4 experiments). Basal media played a larger role in improving neuron health than individual supplement components; cultures maintained in BrainPhys™ retained more attached neurons (% confluence) compared to cultures maintained in unqualified basal media (99.2 ± 0.5%, 40.5 ± 5.8%, respectively; mean ± SEM; n = 4 experiments). Collectively, these results demonstrate that validated media components are key determinants of successful neuronal differentiation. Careful selection of culture media is critical to obtain reproducible and biologically relevant results. Optimized, quality-controlled culture systems such as BrainPhys™ and the STEMdiff™ Forebrain Neuron Kit provide reliable platforms for generating high-quality neuronal cultures suitable for disease modeling and drug screening studies.

Disclosures: G. Yogarajah: A. Employment/Salary (full or part-time): STEMCELL Technologies Inc. **L. Chew:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **S.A. Louis:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **A. Eaves:** A. Employment/Salary (full or part-time): STEMCELL Technologies. **E. Knock:** A. Employment/Salary (full or part-time): STEMCELL Technologies.

Poster

PSTR060: Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR060.02/C24

Topic: B.07. Network Interactions

Support: DFG 326 998 133 - TRR 225 Biofabrication Subprojekt A07

Title: The Role of Astrocytes and Laminins on 3D Cultured Spinal Cord Neurons

Authors: N. MURENU[1], E. TÜRKER[1], M. S. ANDRADE MIER[1], J. WEIGELT[2], A.-L. WIESSLER[1], P. DALTON[3], C. VILLMANN[1], ***N. SCHAEFER**[4];
[1]Inst. for Clin. Neurobio., [2]Dept. of Functional Materials in Med. and Dent. and Bavarian Polymer Inst., Univ. Hosp. Wuerzburg, Wuerzburg, Germany; [3]Dept. of Bioengineering, UO Knight Cmapus, Eugene, OR; [4]Univ. Hosp. Würzburg, Wuerzburg, Germany

Abstract: Complex neurological diseases remain poorly understood due to the limitations of conventional 2D cell culture models, which fail to elucidate the underlying molecular mechanisms. This highlights the need for efficient 3D models to study those neurological diseases, particularly as transgenic animal models are cost-intensive, ethically controversial, and often unsuitable for rare diseases. Creating a 3D spinal cord model for studying neurological diseases presents significant challenges, not only due to the tissue's complexity of neuronal cell types but also its biomaterial requirements such as inherent softness. To address these challenges, our model is based on a MEW-printed PCL scaffold, which serves as a reinforcing structure for the use of ultrasoft hydrogels as a matrix. Primary spinal cord neurons derived from mice are embedded in Matrigel and seeded onto the scaffolds. Comparisons between 2D and 3D neuronal cultures demonstrated the importance of the third dimension in accurately mimicking the in vivo environment, particularly regarding neuronal firing patterns and synaptic activity. The interaction between cellular components and the extracellular matrix is essential for neuronal survival, network formation, and function. Laminins for example play key roles in regulating axon length and facilitating the formation of focal adhesions. In this study, we investigate the effects of distinct laminins on our spinal cord 3D model, as well as the impact of astrocytes for neuronal firing and function. Following analysis of cell-cell interactions and neuronal maturation using immuncytochemical tools and functional analysis by Ca^{2+}-Imaging, we used sera from patients suffering from the neurological disorder stiff person syndrome to evaluate the 3D as suitable to study disease mechanisms. The patient sera harbour autoantibodies against the inhibitory glycine receptor which impact motoneuronal firing. Treating the 3D model with these autoantibodies serves as a proof of concept, weather our model can be used to study molecular pathomechanisms.

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Poster

PSTR060: Network Interactions

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Program #/Poster #: PSTR060.03/C25

Topic: B.07. Network Interactions

Title: Network properties positively correlated with integrated information in neural network models

Authors: *K. NISHIHARA, O. ARAKI;
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Abstract: Our brain constantly receives huge information from the outside world through sensory organs and integrates them appropriately, which results in subjective perception. According to the integrated information theory (Tononi et al., 2008), the integrated information (Φ) defined as minimum information loss based on information theory between naive and divided neural network systems reflects the conscious level quantitatively. Since the calculation cost of Φ is generally very high, alternative evaluation methods for network integration have been studied so far. For example, Global Efficiency (GE), one of the properties of network topology that mainly reflects efficiency of information transfer, was reported to show a positive correlation with Φ (Toker et al., 2019). However, it remains unknown about the correlation between Φ and other network properties. The purpose of this study is to find network properties among some indicators of integrative network structure, which are positively and robustly correlated with Φ in the network models. We examined 8 network properties as follows: GE, Local Efficiency (LE), Clustering Coefficient (C), Degree Standard Deviation (DD), Betweenness Centrality (BC), Closeness Centrality (CC), Eigenvector Centrality (EC), and Fiedler value (Fiedler). As the neural network model whose network structure is close to an actual neural network in terms of higher Φ, we adopted two representative network models, Watts-Strogatz model (WS) (Watts et al., 1998) and Barabási-Albert model (BA) (Barabási et al., 1999). WS models were made from a random network initially using rewiring of edges with a constant probability. For the sake of variety, we did it for 19 different probabilities. On the other hand, BA models were generated by adding nodes and edges, where the wiring probability is proportional to the α power of the degree of each node (α is varied from 0.0 to 3.0). The analysis shows that only the Fiedler have significant positive correlation to Φ in both of WS ($r=0.87$; $p<0.001$) and BA ($r=0.82$; $p<0.001$). Conversely, GE, DD, and CC correlated positively in WS ($r = 0.98, 0.97, 0.95$; $p < 0.001$) but negatively in BA ($r = -0.72, -0.71, -0.71$; $p < 0.001$), whereas BC and EC correlated negatively in WS ($r = -0.89, -0.63$; $p < 0.001$) but positively in BA ($r = 0.73, 0.86$; $p < 0.001$). C and LE showed negative correlations in both models. Fiedler is defined as a second minimum eigenvalue of graph Laplacian matrix $L = D - A$, where A is an adjacency matrix and D is a degree matrix. These results suggest that Fiedler is a prominent candidate for an indicator of neural network integration.

Disclosures: K. Nishihara: None. **O. Araki:** None.

Poster

PSTR060: Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR060.04/C26

Topic: B.07. Network Interactions

Support: NIH Grant NS096092

Title: Region-dependent impact of BDNF on inhibitory signaling from PV cells of the hippocampal formation

Authors: S. ZHANG[1], *G. MACCAFERRI[2];
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Abstract: Parvalbumin-expressing interneurons (PVs) exert fast control on the cellular excitability of their targets via GABAa receptors. Synaptic signaling mediated by PVs in the hippocampal formation is necessary for healthy computations, and its disruption may lead to altered information processing and/or epileptiform activity. We have previously shown (Maccaferri, 2024) that the application of the neurotrophin brain-derived-neurotrophic-factor (BDNF, 3nM for 1.5 hour) to subicular slices alters PV network signaling and turns physiological inhibitory synaptic output onto pyramidal cells (PCs) into a complex biphasic event, mediated (early) by monosynaptic GABAa receptors followed by a (late) polysynaptic excitation via glutamate receptors. We have performed additional whole-cell recordings and further studied the responses of PCs to the optogenetic stimulation of PVs in BDNF-treated subicular slices. The aforementioned transformation was prevented by the TrkB antagonist ANA-12 (100 uM, n=53 PCs), thus indicating that it was triggered by binding of BDNF to TrkB receptors. Also, we have found that this phenomenon is attenuated by the exposure of slices to the putative KCC2 activator CLP 257 (30 uM, n=13 PCs), which supports our original interpretation of BDNF/TrkB-dependent downregulation of the KCC2 transporter, leading to excitatory GABAergic signaling and activation of polysynaptic glutamatergic pathways. A prediction of this interpretation is that this phenomenon should also depend on the level of reciprocal interconnectivity between glutamatergic cells, which, within the hippocampal formation, is highest in the subiculum (Fiske et al., 2020). Therefore, we tested the effects of the same BDNF treatment on granule cells (GCs) of the hippocampal dentate gyrus that do not establish reciprocal connectivity. As expected, no late excitatory phase was observed in response to the stimulation of PVs either in control (n=31 GCs) or BDNF-treated slices (n=32 GCs). In conclusion, our results highlight the synergistic effect of BDNF/TrkB-dependent downregulation of the KCC2 transporter and local excitatory connectivity in setting the vulnerability of cortical networks to information processing disruption and, potentially, in initiating pathological epileptiform activity.

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Poster

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Title: Medial entorhinal layer 2 stellate cell activity is sufficient to drive type 2 dentate spikes and pathological hypersynchronous events in healthy and epileptic mice

Authors: *P. A. PHILIPSBERG[1], E. COLEMAN[1], Z. CHRISTENSON WICK[1], Y. FENG[1], K. PIMSHAN[1,2], C. KOHLER[1], C. G. KENTROS[3], T. SHUMAN[1];
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Abstract: Type 2 dentate spikes (DS2s) are synchronizing events thought to be important for spatial memory in the healthy brain. Medial perforant path projections have long been thought to drive DS2s, however the specific contribution of medial entorhinal layer 2 (MEC2) stellate cells to DS2s has never been experimentally tested. Further, the extent to which mechanisms driving dentate spikes in heathy animals are coopted to produce pathological hypersynchronous events in epileptic animals remains unknown. Here we characterize dentate spikes and MEC2 excitatory firing in both healthy and epileptic mice, and optogenetically activate MEC2 stellate cells to elicit DS2-like events and pathological interictal epileptiform discharges (IEDs). We used dual-region silicon probe recordings in MEC and hippocampus to examine neural activity during dentate spikes in both heathy and chronically epileptic mice. We expressed ChR2 specifically in MEC2 stellate cells in order to optogenetically identify them and directly investigate their role in initiating DS2s. We found that the firing rates of MEC2 excitatory cells increase preceding a DS2 and that this modulation of firing rate is increased in epileptic mice, which also have higher rates of DS2s. Further, we show that optotagged MEC2 stellate cells increase in firing rate more than non-optotagged MEC2 excitatory neurons. Finally, we show that activating MEC2 stellate cells can elicit DS2-like events, while stimulating with higher light power can drive IEDs even in control animals. These results provide new evidence that MEC2 stellate cell activity contributes both to physiological DS2s and is sufficient to induce pathological hypersynchronous events in epilepsy. This provides a novel therapeutic target for treating hypersynchrony contributing to seizures and cognitive impairments in temporal lobe epilepsy.

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Poster

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Topic: B.07. Network Interactions

Support: iBehave Neuroscience Network funded by the state of North-Rhine-Westphalia, Germany German Research Foundation, GR4310-11/1

Title: Age-dependent changes in whole-brain calcium wave propagation in Drosophila

Authors: *I. GUATIBONZA AREVALO[1], K. Y.-C. CHENG[2], S. AIMON[3], A. MÚNERA[4], I. C. GRUNWALD KADOW[5];
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Abstract: Calcium dynamics are a fundamental component of the brain's signaling architecture, playing essential roles in neuronal communication and network coordination. Aging, as a long-term physiological process, induces widespread alterations in the brain, including changes at the molecular, cellular, and circuit levels. In this study, we investigate how aging affects the dynamics of a spontaneous and induced global calcium wave event in the adult *Drosophila* brain. We quantified calcium dynamics from 2D and 3D in-vivo whole brain calcium recordings of adult *Drosophila* expressing pan-neuronal GcaMP using an optic flow-based method. We compared spontaneous and KCl-induced global calcium waves in young (0-3 days) and aged (17-25 days) flies. Subjects were mounted for stable whole-brain imaging preserving partial mobility. Selected 3D datasets were reconstructed for studying wave propagation. Spontaneous waves were detected post-dissection, while induced waves were elicited by 100 mM KCl, applied either 0h or 2 h after dissection. Velocity vector fields were estimated using the Lucas-Kanade optical flow algorithm, which allows robust local displacement tracking in temporally-resolved fluorescence data. Complex propagation patterns and critical points were identified and classified using a Jacobian-based analysis of the flow fields, including sources, sinks, spirals, and saddles. We found that aging was associated with increased probability of observing spontaneous calcium waves. In older flies, these spontaneous waves exhibited a smaller change in fluorescence intensity compared to younger flies, and longer post-dissection times were associated with an increased presence of multiple high-frequency components within the wave event. In contrast, young flies power at lower frequencies with longer post-dissection times. In both age groups, frequencies between 1 and 40 x 10^{-3} Hz were consistently found in spontaneous waves. Application of 100 mM KCl reliably induced calcium waves in both age groups. When applied immediately after dissection, waves showed large calcium fluctuations with low-frequency oscillations. When applied after 2h post-dissection, sustained calcium waves displayed dampened oscillatory dynamics. These observations suggest that while propagation related mechanism is preserved with age, the regulatory dynamics governing subsequent oscillations is sensitive to tissue’s physiological condition. Moreover, the spatial propagation pattern and the

distribution of critical points appear to be age-dependent, possibly reflecting age-related changes in signaling architecture.

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Poster

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Program #/Poster #: PSTR060.07/C29

Topic: B.07. Network Interactions

Title: Cortical interneuronal circuitry inferred from single-cell RNA-seq.

Authors: *L. HEINLY[1], P. NARNUR[2], A. SCHULMANN[3], Y. PATEL[4], D. KIM[5], P. K. AULUCK[1], S. MARENCO[1];
[1]Human Brain Collection Core, Natl. Inst. of Mental Health, Natl. Inst. of Hlth., Bethesda, MD; [2]Dept. of Computat. Biomedicine, Boston Univ. Med. Ctr., Boston, MA; [3]Dept. of Psychiatry, Columbia Univ. Irving Med. Center, New York State Psychiatric Inst., New York, NY; [4]Univ. of Toronto, Toronto, ON, Canada; [5]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Background: Cell-to-cell communication (CCC), through interactions between ligands and cognate targets (interaction pairs), is the fundamental basis for neuronal activity. Single-cell RNA sequencing (scRNA-seq) enables the identification of cell types and the ligands and receptors they express. Though interaction pair connectivity is not necessarily synaptic, it allows to infer local connectivity patterns among neurons. We focused on interneurons as they are conserved across neocortical regions and their connectivity is local. **Methods:** We used the Yao et al. 10X dataset (2021) of 13 adult mouse cortical regions (ROIs), excluding ROIs with fewer than 3 subjects, leaving 7 ROIs represented by 29 subjects. CellChat (Jin et al., 2021) was used to calculate CCC probability for each interaction pair, focusing on 8 interneuron cell types (LAMP5, LAMP5LHX6, SNCG, VIP, PVALB, CHAND, SST, SST-CHODL). We measured the variance in CCC explained by cell type pair, subject, or ROI. We used principal component analysis (PCA) to cluster cell type pairs by interaction pairs with the expectation that connected cell types would form discrete clusters. Clusters were evaluated and categorized based on sender-receiver cell type pair. **Results:** Median %variance explained by cell type pair was 14%, while subject and ROI contributed < 2% and < 1% of the variance respectively, confirming that cell type pair was the main driver for CCC. PCA identified 19 clusters demonstrating discrete connectivity between all interneuron cell types. LAMP5 and LAMP5LHX6 always appeared together as senders and receivers, indicating that these cell types do not differ in their connectivity to other interneurons. All clusters but one had a single sender cell type ("sender-driven"). CHAND cells had the most diffuse connectivity, projecting to all other types except for LAMP5/LAMP5LHX6 in one cluster. LAMP5/LAMP5LHX6 had the most specialized connectivity appearing as the single sender in 4 clusters and as the single receiver in 5. Medial

ganglionic eminence derived cell types (PVALB, SST, SST-CHODL, CHAND) combined as receivers in 7 clusters, while caudal ganglionic eminence derived types (VIP, SNCG, LAMP5) combined in 6. **Conclusion:** We developed an interaction pair defined connectivity map that describes the dense circuitry between interneuron cell types, consistent with previous functional studies. Notably, CHAND and LAMP5 cell types have radically different patterns of connectivity to other cell types, suggesting distinct regulatory roles in local CCC.

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Poster

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Topic: B.07. Network Interactions

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Title: Scale-free, non-linear responses to holographic perturbation in the visual cortex of awake mice

Authors: *T. L. RIBEIRO, S. PAJEVIC, B. GIFFORD, D. PLENZ;
NIH, Bethesda, MD

Abstract: One fundamental aspect of the brain's function is its ability to detect novel, unanticipated events and rapidly relay this information across relevant areas, thus enabling effective behavioral responses. Critical brain dynamics theory predicts a non-linear, scale-free response to such perturbation nearby a second-order phase transition on such networks. To further understand how novel spikes generated in local groups of neurons propagate through the network, we combined a low-repetition, high-power laser (Carbide/Orpheus; LightConversion) and a high-resolution spatial light modulator (MeadowLark), achieving successful 2-photon (2P) excitation of individual neurons expressing the opsin ChrimsonR in the awake mouse. Simultaneously, we imaged layer II/III pyramidal neurons of widefield-identified primary visual cortex (V1) expressing GCaMP8s. The activity of ~150 - 300 neurons was recorded using 2P imaging in a ~450 x 450 μm area (100 - 200 μm depth) at ~45 Hz framerate while holographically stimulating (100 ms; <10 mW per target) 32 pyramidal neurons co-expressing the opsin (~100 trials per group). The chosen 32 targets were grouped together for stimulation in subsequent recordings, from group size of 1 neuron to 2, 4, 8, 16 and 32. The groups were formed hierarchically, by consecutively merging groups that are spatially close. Images were denoised using a machine-learning-based algorithm and denoised calcium traces were then deconvolved for spike extraction. During stimulation of the target cells, a subset of non-stimulated cells responded significantly to the perturbation (> 92% baseline spike count), with a

bias towards neurons with similar orientation preference as the targets. Population responses to the perturbation increased non-linearly with input size. To better understand our experimental results, we employed numerical simulations of cellular automata 2D sparse networks, which could be tuned in and out of criticality. By mimicking our experimental recordings and driving target sites on these in silico networks and applying the same analysis we performed for the experimental data, we obtained similar results only at the critical point, suggesting the observed responses on the brain networks are generated by and benefit from being close to criticality.

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Poster

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Topic: B.07. Network Interactions

Support: R01AG084036
1 U01 AG076804-01

Title: Neural Network Architecture of the Mouse Basal Pontine Nuclei

Authors: *Q. ZHAO[1], B. ZINGG[2], M. RUDD[1], J. SUN[1], H. DONG[2];
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Abstract: The basal pontine nuclei (BPN), positioned between the cerebral cortex and cerebellum, serve as a critical relay in the cortico-pontine-cerebellar system—one of the brain's largest projection networks. The BPN's complex, clustered organization is thought to mediate the transformation from topographically organized cortical inputs to the distributed representations observed in the cerebellar cortex. While this system has been extensively studied in rats, a comprehensive wiring diagram in the mouse brain remains lacking. In this study, we aim to construct a brain-wide connectivity atlas of the cortico-pontine-cerebellar loop in the mouse. Using single and multiple tracer injections into defined cortical, pontine, and cerebellar regions, we map both anterograde and retrograde projections to delineate region-specific input-output relationships. This work will reveal the spatial organization and structural logic of this network, providing novel insights into how the BPN integrates multimodal information and contributes to sensorimotor processing and behavior at cellular and systems levels.

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Poster

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Topic: B.07. Network Interactions

Support: NIH

Title: Computational Model of Electrical Stimulation in the Dentate Gyrus and the Downstream Effects on Entorhinal Cortex Axons and Granule Cells Activation

Authors: *T. WEI[1], S. FARZAD[2], J.-M. C. BOUTEILLER[3], G. LAZZI[1];
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Abstract: As a key structure for memory formation and spatial navigation, the hippocampus is critical to daily life but is vulnerable to disorders such as Alzheimer's disease and epilepsy. Electrical stimulation has emerged as a promising strategy to restore or modulate hippocampal function. To investigate how stimulation parameters influence neuronal activity, we developed a large-scale computational model encompassing the dentate gyrus (DG), entorhinal cortex (EC) axons, and granule cells (GCs). Bipolar electrodes were placed along the crest edge of the DG to deliver biphasic pulses of varying amplitudes. The 2D spatial distribution of EC axon locations was mapped to 3D coordinates, and morphologies were reconstructed using the ROOTS Python library. Biophysical properties were inserted into EC axons to create a functional model of their electrophysiology. A connectivity map was derived from statistical properties to link EC axons to GCs. We implemented the Admittance-Method to compute extracellular voltages within the voxelized DG under stimulation, and interpolated these voltages to each EC axon segment. These values were applied to the functional EC axon models in NEURON to simulate action potential generation. The resulting EC axon spikes were then used to drive synaptic inputs onto GCs, enabling us to capture the spatiotemporal activation patterns of both populations. Our results indicate that EC axons located farther from the center of the bipolar electrodes exhibit delayed activation compared to those closer to the center. Furthermore, as the stimulation amplitude increases, the activation rate of both EC axons and granule cells also rises. Specifically, while 100 nA stimulation shows activation in these populations, 150 nA results in a broader activation profile. However, when the amplitude is further increased to 200 nA, overall activation begins to decline, indicating a potential inhibitory effect at higher stimulation levels. These findings suggest the existence of an upper threshold beyond which excessive current may suppress neuronal activity rather than enhance it. This highlights the importance of selecting an optimal stimulation amplitude to effectively engage hippocampal circuits, and provides valuable insight for the design of neuro-modulatory medical devices aimed at maximizing activation efficiency.

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Poster

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Topic: B.07. Network Interactions

Support: Italian Ministry of Health Grant GR-2021-12372496

Title: Diabetes alters the expression and regional distribution of perineuronal nets in the central nervous system

Authors: S. CASTALDI[1], S. NOTARTOMASO[2], A. CECCHERELLI[1], R. GINERETE[1,2], D. BUCCI[2], F. LIBERATORE[2], T. IMBRIGLIO[2], M. ALBORGHETTI[1,2], M. CANNELLA[2], G. BATTAGLIA[1,2], V. BRUNO[1,2], *F. NICOLETTI[1,2], **G. MASCIO**[2];
[1]Univ. Sapienza, Roma, Italy; [2]I.R.C.C.S. Neuromed, Pozzilli, Italy

Abstract: Diabetes mellitus is consistently associated with CNS complications, including cognitive dysfunction and alterations in sensory processing. The underlying mechanisms are only partially known and may include microvascular abnormalities, neuroinflammation, and changes in the extracellular matrix (ECM). These mechanisms may ultimately result into maladaptive changes in synaptic plasticity and network activity. Perineuronal nets (PNNs), which are condensed aggregates of ECM enwrapping parvalbumin (PV)-positive GABAergic interneurons in most forebrain regions, play a key role in the regulation of cortical network activity by shaping the dynamic interplay between interneurons and principal neurons. PNNs are formed by complex proteoglycans, and, therefore, it is intuitive that their expression might be altered as a result of the profound changes in glucose metabolism occurring in diabetes mellitus. Here, we used the streptozotocin model of type-1 diabetes in mice to examine brain region-specific changes in PNN density and composition as related to cognitive function, anxiety-like behavior, and behavioral responses to painful stimuli. Mice with blood glucose levels > 250 mg/dl in response to a single injection of streptozotocin (200 mg/kg, i.p.) were selected for immunohistochemical, biochemical, and behavioral analysis. To our surprise, changes in PNN density were highly heterogenous in different brain regions of diabetic mice, with *Wisteria Fluribunda Agglutinin* (WFA)-positive PNNs being markedly reduced in the somatosensory cortex and medial prefrontal cortex, and significantly increased in the insular cortex, reticular thalamic nuclei, and amygdala. Interestingly, biochemical and cellular markers of neuroinflammation, such as RAGE, Iba-1, and CD68, were increased in the somatosensory and medial prefrontal cortex, but not in the insular cortex, suggesting that local inflammation may drive the loss of PNNs, as a consequence of an enhanced PNN degradation or other mechanisms. Diabetic mice showed a reduction in mechanical and thermal pain thresholds, as a result of peripheral neuropathy, and pain thresholds were unaffected by enzymatic degradation of PNNs in the insular cortex. In contrast, diabetic mice showed a greater tolerance to pain (assessed with the place escape/avoidance paradigm test) and an improvement in social cognition after PNN depletion in the insular cortex. Our findings demonstrate for the first time that diabetes causes substantial changes in PNN density in different forebrain regions, and that these changes may contribute to some of the CNS complications of diabetes mellitus.

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Poster

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Program #/Poster #: PSTR060.12/C34

Topic: B.07. Network Interactions

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McKnight Technology Award 2019

Title: Large-scale Visualization of Ca^{2+}Signals and Network Activity in the Suprachiasmatic Nucleus (SCN)

Authors: *M. ZURSIN, S. A. MCGRAW, K. C. BENTON, J. A. EVANS, I. A. SAVTCHOUK;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: How memories are processed and consolidated by neural networks is perhaps the greatest remaining mystery of modern neuroscience. Neocortical networks are required for encoding and retrieving long-term memories, and much is known about the neural ensembles and synaptic mechanisms encoding plasticity in these laminar structures. In contrast, much less is understood about mechanisms by which non-laminar brain areas in the hypothalamus and brain stem process and encode stimuli that induce plasticity. One hypothalamic structure where plasticity is essential for network function is the central circadian clock in the suprachiasmatic nucleus (SCN). To coordinate physiology with the solar cycle, the SCN is reset daily by light inputs that adjust the circadian molecular clock. SCN responses to light are influenced by stimulus properties as well as circadian oscillations in the state of the network. Thus, the effects of light on circadian clock function vary with time of day, but network mechanisms causing this form of metaplasticity remain ill-defined. Further, the SCN is a heterogenous network composed of GABAergic neurons that differ in anatomical location, molecular identity, and oscillatory properties. Understanding how these neuronal clusters process light and interact to adjust the circadian program has proven challenging due to the dynamic oscillations and tortuous connections of this network. To better understand the dynamic process by which SCN sub-networks process light to remodel the circadian template, we performed fast 3D two-photon imaging in acute slices of adult mice expressing calcium-sensitive reporter (GCaMP). Changes in calcium levels were quantified to extract features (e.g. amplitude, duration, latency) of their responses to stimulation. Further, we used unsupervised machine learning (ML) approaches to cluster cells into functional subgroups. The resulting patterns were then mapped back to the parent SCN sub-regions hosting the originating cells. To detect the circadian template ensembles, we developed custom Python-based software that allowed exploration and visualization of the

extracted response features as well as the resulting spatiotemporal patterning of responses across the SCN network. Software capabilities include user-controllable selection, filtering, and grouping of cells based on their activity, evoked responses, and other customizable criteria. Using this approach, we identified sub-groups of cells within the SCN with functionally distinct responses to the inputs. This may lead to better understanding of how information flow is shaped by dynamic ensembles within non-laminar networks.

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Poster

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Program #/Poster #: PSTR060.13/C35

Topic: B.07. Network Interactions

Title: Large-scale 3D EM connectomics dataset of mouse hippocampal area CA1

Authors: *M. CORTEZE[1], E. GRASSO[2], H. SCHMIDT[3];
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Abstract: The hippocampal formation is thought to be crucial for memory and learning, with subarea cornu ammonis 1 (CA1) considered to play a major role in spatial and episodic memory formation, and for evaluating the match between retrieved memories and current sensory information. While enormous progress has been made in classifying CA1 neurons based on molecular, morphological, and functional properties, and in identifying their role in behavioral tasks, a clear understanding of the underlying circuits is still missing. Here, we present the first large-scale three dimensional (3D) electron microscopy dataset of mouse CA1 at nanometer-scale resolution. The dataset is available online and can be readily used for circuit reconstructions, as demonstrated for inputs to CA1 superficial layers. Example volume segmentations show that automated reconstruction detection is feasible. Using these data, we find evidence against the long-held assumption of a homogeneous pyramidal cell population. Furthermore we find substantial possibly long-range axonal innervation of stratum-lacunosum interneurons, suggested previously to originate in L2 of MEC. These first analyses illustrate the usability of this dataset for finally clarifying the connectomic properties of mouse CA1, a key structure in mammalian brains.

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Poster

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Topic: B.07. Network Interactions

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Title: App and dna damage create a feedforward loop that contributes to alzheimer's disease pathogenesis

Authors: *F. MA, F. STAUFFER, M. BAGNELL, R. KINGSTON, S. WELTY, G. GUTTA, E. BRENNER, H. AKOLKAR, K. HERRUP;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Alzheimer’s disease (AD) is a devastating neurodegenerative disease with unknown pathogenesis and no known effective therapeutic options. The amyloid precursor protein (APP) has occupied a central position in the pathophysiological description of AD because its proteolytic product, the β-amyloid (Aβ) peptide, is the main contributor to the amyloid plaques that are hallmarks of AD. Aβ-independent roles for the full-length APP protein in AD, however, are relatively understudied. We have approached this problem from the perspective that APP is recognized as a damage-response protein that is elevated after a variety of different stresses. Our current work demonstrates that DNA damage, in and of itself, is sufficient to induce APP expression. This takes on added significance since others have shown that increased neuronal activity also leads to transient DNA damage that is quickly repaired. We explored this connection both directly, by inducing DNA damage with etoposide, and indirectly by blocking DNA repair. The latter was achieved by blocking ATM (ataxia-telangiectasia mutated) both pharmacologically and genetically. In each situation, enhanced DNA damage led to elevated levels of APP. The linkage between APP and DNA damage appears to be bi-directional as we found that an increase in APP is sufficient to cause DNA damage. In AD brains of both mouse and humans, where APP levels are elevated, DNA damage was significantly increased. Further, transfection of wild type neurons with plasmids that overexpress APP led to significant DNA damage. This damage was blocked by application of a BACE1 inhibitor (verubecestat) or a γ-secretase inhibitor L685458. These results would appear to support a key role for the Aβ peptide. Application of oligomeric Aβ directly to neurons, however, resulted in no significant increase in DNA damage. This led to the realization that both verubecestat and L685458 would also inhibit the production of the APP intracellular domain (AICD) and transfection of AICD encoding plasmids is sufficient to induce DNA damage. The findings link DNA damage, a hallmark of aging, and APP, a hallmark of AD. The linkage appears to be bi-directional and paints the picture of a pathway with the potential to become a dangerous feed-forward loop. Our findings reveal a novel role for full length APP in AD pathogenesis through its relationship with DNA damage.

Disclosures: F. Ma: None. **F. Stauffer:** None. **M. Bagnell:** None. **R. Kingston:** None. **S. Welty:** None. **G. Gutta:** None. **E. Brenner:** None. **H. Akolkar:** None. **K. Herrup:** None.

Poster

PSTR060: Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR060.15/C36

Topic: B.07. Network Interactions

Support: DARPA ABC Grant
Boehringer Ingelheim Fonds PhD Fellowship

Title: Whole-brain imaging in larval zebrafish to understand neural mechanisms of general anesthesia

Authors: *M. DUQUE RAMIREZ[1], N. HOERMANN[2], K. J. HERRERA[3], F. ENGERT[4], M. B. AHRENS[5];
[1]Harvard Univ., Cambridge, MA; [2]Harvard Univ., Boston, MA; [3]SCRB, [4]MCB, Harvard Univ., Cambridge, MA; [5]Janelia Res. Campus / HHMI, Ashburn, VA

Abstract: Understanding how anesthetic agents differentially affect neural circuits and vital functions is crucial for optimizing both safety and efficacy. We present a larval zebrafish platform that integrates high-speed dual-color light-sheet calcium imaging with simultaneous cardiac monitoring to probe the mechanistic basis of anesthesia. By comparing ketamine, propofol, and several novel experimental candidates, we performed dose-response behavioral assays alongside real-time heart-rate recording to establish each agent's safety profile. Volumetric imaging captured whole-brain activity dynamics during induction and emergence, revealing that distinct neuronal circuit groups are differentially suppressed or preserved depending on the anesthetic. Concurrent cardiac readouts allowed us to correlate neural suppression patterns with bradycardia risk. Although specific circuit identities remain to be fully defined, preliminary observations suggest that agents which preserve activity in key arousal-related networks maintain autonomic stability. Additionally, dual-color imaging enabled us to begin exploring potential glial contributions to network reconfiguration during recovery. Collectively, this integrated approach yields a rapid, physiologically relevant assay for dissecting how various anesthetics modulate neural and autonomic states. Such mechanistic insights will inform the rational design of next-generation agents that strike an optimal balance between unconsciousness and safety.

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Poster

PSTR060: Network Interactions

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Program #/Poster #: PSTR060.16/C37

Topic: B.07. Network Interactions

Title: Interplay between MOR1 and GABAergic signaling in the morphine-induced hyperalgesia and tolerance

Authors: *N. KORNEEVA[1,2], R. FAN[1], C. WILTZ[3], S. ABURAHMA[2], P. BRACEY[1], E. BROWN[1], T. ARNOLD[1];
[1]Emergency Med., [2]Pharmacology, Toxicology and Neurosci., [3]Grad. Sch., LSUHS-Shreveport, Shreveport, LA

Abstract: Nadejda Korneeva[1,2], Ruping Fan[1], Christopher Wiltz[2], Suliman Aburahma[2], Paul Bracey[1], Elaine Brown[1], and Thomas Arnold[1] [1]Department of Emergency Medicine; [2]Department of Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center; Shreveport, LA 71130-3932 Opioids are the most effective drugs commonly prescribed to treat pain. Sex differences are documented for the rates of opioid-related overdoses, abuse patterns, and drug-induced physiological effects. In our previous study, we observed that female mice display higher anti-nociceptive and motion responses to acute morphine administration than male mice. However, male mice developed tolerance to chronic morphine administration faster compared to that in female mice. Both male and female mice developed hyperalgesia after 30-days of morphine administration. We have investigated the distribution and activity of MOR1 and GABAAR in mouse brains after 12 and 30 days of morphine treatment. We observed the opposite trends in the levels of MOR1 and GABAAR in mouse stem and cortex areas after chronic morphine administration. Moreover, the basal levels of these receptors were different between males and females, suggesting different mechanisms of morphine-induced hyperalgesia/tolerance between males and females. In our previous study, we demonstrated that chronic morphine administration increases the level of BDNF in male and female mouse brains with male brains containing higher BDNF expression. BDNF is involved in the drug-adaptation processes with a possibly higher effect on males compared to females. We will present the trends in MOR1, GABAAR, and BDNF signaling after acute and chronic morphine administration in mice, and discuss their differential effect on the development of hyperalgesia/tolerance in males and females.

Disclosures: N. Korneeva: None.

Poster

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Topic: B.07. Network Interactions

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NSF grant 2123971
S10 MH124733–01

Title: Mapping Brain-Wide Connectivity in a CaV3.3 Mutant Mouse Model of Schizophrenia Using Awake 14T fMRI

Authors: N. PASUPATHY[1], C. REILLY[1], W. XU[1], X. YU[1], J. Q. PAN[2], ***X. A. ZHOU**[1]; [1]Harvard Med. School/MGH, Boston, MA; [2]Stanley Ctr. for Psychiatric Res., Broad Inst., Acton, MA

Abstract: Introduction
Disrupted thalamocortical communication is a core characteristic of schizophrenia, frequently marked by reduced sleep spindle activity originating in the thalamic reticular nucleus (TRN). Loss-of-function variants in the CaV3.3 (CACNA1I) T-type calcium channel, highly expressed in TRN GABAergic neurons, have been linked to schizophrenia risk. However, the impact of CaV3.3 dysfunction in TRN on brain-wide dynamics remains poorly understood. Here, we used high resolution multimodal imaging in a mouse model carrying the schizophrenia-associated CaV3.3 R1305H mutation selectively in TRN neurons.
Methods
RH mutant mice and WT littermates ((n = 3 for each group, 25-30g, 3-6 months old) were implanted with a 600 MHz single-loop RF coil fixed over the skull. Following recovery, mice underwent 5 weeks of acclimation to awake head-fixed imaging. High-resolution resting-state fMRI in awake mice was acquired at 14T using a 2-segment multi-slice 2D EPI sequence (100x100x200 μm, TR = 1s, TE = 7ms, matrix =144 × 96). T1-weighted anatomical scans were used for registration to a standard mouse atlas. Seed-based ROI analyses were performed using the anterior cingulate cortex (ACC), barrel cortex (BC), and visual cortex (VC) as seeds.
Results
Resting-state BOLD maps revealed that RH mice showed increased functional connectivity from ACC, BC, and VC compared to WT littermates. This was confirmed by voxel-wise group comparisons ($p < 0.05$ uncorrected, cluster size > 40). Increases were widespread, suggesting elevated cortex-wide low-frequency synchrony in RH mice.
Discussion
Our findings suggest that CaV3.3 dysfunction in TRN GABAergic neurons alters resting-state brain dynamics, likely by reducing thalamic inhibition and increasing slow cortical synchrony. This network disorganization may contribute to schizophrenia-related cognitive deficits. By combining genetic specificity with awake high-field fMRI, this study provides a framework for investigating thalamocortical disinhibition in psychiatric disorders.
Future Directions
Ongoing studies are expanding this framework by increasing fMRI sampling rate and incorporating in vivo calcium photometry to directly link TRN activity, spindle dynamics, and functional connectivity across sleep and sensory states.

Disclosures: N. Pasupathy: None. **C. Reilly:** None. **W. Xu:** None. **X. Yu:** None. **J.Q. Pan:** None. **X.A. Zhou:** None.

Poster

PSTR060: Network Interactions

Location: SDCC Halls B-H

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Program #/Poster #: PSTR060.18/C39

Topic: B.07. Network Interactions

Title: Basal forebrain control of large-scale fMRI networks

Authors: *V. BEDIN[1,3,2], S. GINI[3,2], A. GALBUSERA[3], A. GOZZI[3];
[1]Univ. of Trento, TRENTO, Italy; [2]Ctr. for Mind and Brain Sci., Univ. of Trento, Rovereto, Italy; [3]Functional Neuroimaging Laboratory, Ctr. for Neurosci. and Cognitive Systems, Italian Inst. of Technol., Rovereto, Italy

Abstract: Resting-state fMRI (rs-fMRI) enables the identification of brain regions exhibiting synchronous fluctuations in spontaneous BOLD signals. Extensive research has shown that the resulting patterns of synchronous activity, often referred to as resting-state networks (RSNs), are evolutionarily conserved and undergo structured dynamic reconfiguration as a function of brain state. However, the mechanisms orchestrating the dynamic organization of RSNs remain elusive. Neuromodulatory systems such as the basal forebrain (BF) - a major source of cortical acetylcholine and a key regulator of arousal - are strong candidates for controlling RSNs dynamics. Yet, whether and how BF output causally organizes large-scale RSNs is unclear. Here, we addressed this question by employing targeted manipulations of the BF in awake, head-fixed mice. To probe the effect of global BF output on RSNs dynamics, we induced pan-neuronal lesions in the BF via local expression of diphtheria toxin. To do so, we leveraged a genetically modified mouse line (ROSA-DTA) in which expression of the diphtheria toxin is Cre-dependent. By injecting Cre-expressing viral vectors directly in the BF, we triggered selective neuronal ablation in the targeted area. In a subsequent study, we expressed excitatory DREADDs in the BF of Pv-Cre mice via viral injections. By using chemogenetics, we assessed the selective contribution of BF parvalbumin-positive (Pv+) neuronal population to the control of RSNs organization. The outcomes of these manipulations were investigated using rs-fMRI. Chronic BF ablation produced widespread reconfiguration of fMRI connectivity, with robustly strengthened integration within the default mode network (DMN) and a prominent reduction in connectivity between cortico-cortical systems. In contrast, activation of BF Pv+ neurons strongly increased cortico-cortical coupling, resulting in enhanced connectivity between the DMN and somatomotor networks. These effects were brain state dependent, as they were strongly attenuated and qualitatively different in states of low arousal induced by sedation. This indicates that arousal critically gates how the BF modulates RSN dynamics. Together, our results provide initial evidence that the BF exerts a causal, arousal-dependent influence on RSNs. Moreover, they suggest that distinct BF cell populations, such as Pv+ neurons, might specifically influence

cortical networks dynamics. Ongoing studies are exploring the roles of additional BF neuronal subtypes in orchestrating RSNs configuration.

Disclosures: V. Bedin: None. **S. Gini:** None. **A. Galbusera:** None. **A. Gozzi:** None.

Poster

PSTR060: Network Interactions

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Program #/Poster #: PSTR060.19/C40

Topic: B.07. Network Interactions

Support: NIH R01 MH118344
Swebilius Foundation

Title: Comparative analysis of synchronized network activity in an epilepsy brain organoid model

Authors: *C. LO[1], J. KIM[2], M. SCANDURA[3], I. H. QURAISHI[4], I.-H. PARK[5];
[1]Yale university, New Haven, CT; [2]; [3]Yale Univ., New Haven, CT, ; [4]Yale Univ., NEW HAVEN, CT, ; [5]Yale Univ., New Haven, CT

Abstract: The intricacies of functional network development in the brain is a key distinguishing trait of humanity yet very difficult to model from human cells. Brain organoids developed from human stem cells fill this gap. I utilize organoids to study defects in the neurodevelopment of functional networks in the developing brain and how it differs in epilepsy. One epilepsy syndrome caused by a rare mutation in *NACC1* (R298W) was recently discovered that also includes neurodevelopmental delay, feeding difficulties, congenital cataracts, and delayed myelination. Using a mutant and control hESC line (H9) with the R298W mutation, I show that organoids with the mutation have significant differences in network activity and functional connectivity. I generated cortical and medial ganglionic eminence organoids and fused them to create assembloids with both excitatory and inhibitory neurons. At day 90, I performed calcium imaging using gCAMP6s over several sessions (n=5) and found significant differences in the activity of the mutant and control organoid. In addition to differences in individual measures such as event count and amplitude, I characterized synchrony of the overall network using different correlation coefficients and synchronization during calcium transient events. Finally, I present functional and spatial maps of connectivity in different fields of view to demonstrate diversity of network formation within the organoid. These findings reflect network phenomenon that occur during epileptogensis found in brain slice studies, demonstrating its utility for understanding disease. Brain organoids derived from human cells are a powerful tool to study neurogenesis and morphological differences, but functional characterization has been limited. This work demonstrates an analytical pipeline to understand functional network formation in organoids, critical in diseases such as epilepsy and autism. On a broader scope, these findings

show how organoids can fit within the overall field that includes older in vitro neuroscience work.

Disclosures: C. Lo: None. **I. Park:** None.

Poster

PSTR060: Network Interactions

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Program #/Poster #: PSTR060.20/C41

Topic: B.07. Network Interactions

Support: Canadian Institutes of Health Research 426485

Title: The anterior cingulate cortex drives lateralized age-dependent inhibition of the claustrum

Authors: T. SHAKER[1], M. ALMOKDAD[2], L. RAZENBERG[3], M. M. KARNANI[4], ***J. C. JACKSON**[2];
[1]Physiol., [2]Univ. of Alberta, Edmonton, AB, Canada; [3]CNCR, VU Univ., Amsterdam, Netherlands; [4]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: The anterior cingulate cortex (ACC) signals top-down information to several cortical and subcortical brain regions. ACC connections with the claustrum are thought to be important for salience detection, cognitive control, and pain processing. However, the circuit mechanisms underlying ACC-claustrum communication remains incompletely understood. We made in vivo single neuron recordings in the claustrum to determine how ACC inputs control claustrum excitatory and inhibitory neurons in neonatal, juvenile, and adult mice. In adulthood, ACC inputs primarily evoked net inhibition of the ipsilateral claustrum and activation of the contralateral claustrum. However, excitatory cell activation in both claustrum hemispheres was sublinear due to the strong feed-forward activation of interneurons that constrained claustrum excitatory neuron firing. In neonatal mice, claustrum parvalbumin interneurons were absent for the first two weeks of life. Consequently, ACC activation drove the feed-forward excitation of claustrum excitatory cells early postnatally which subsequently matured to inhibition across the third postnatal week. Therefore, ACC-claustrum circuits undergo a switch from feed-forward excitation to inhibition and ultimately settles on a hemispheric imbalance favoring ACC excitation of the contralateral hemisphere in adulthood. This ACC-claustrum circuit organization may provide an efficient route for the ACC to modulate the contralateral cortex while minimizing claustrum-cortex interference in the ipsilateral hemisphere.

Disclosures: T. Shaker: None. **M. Almokdad:** None. **L. Razenberg:** None. **M.M. Karnani:** None. **J.C. Jackson:** None.

Poster

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Topic: B.07. Network Interactions

Support: Canadian Institutes of Health Research 426485

Title: Topographic organisation of the claustrum-amygdala-prefrontal circuitry in the common marmoset (Callithrix jacchus)

Authors: *B. HOANG[1], D. WRIGHT[4], A. C. ROBERTS[5], M. G. ROSA[2], D. H. RESER[3]; [1]Monash Univ., Melbourne, Australia; [2]Dept. of Physiol. and Biomedicine Discovery Inst., [3]Monash Univ., Clayton, Australia; [4]Central Clin. Sch., Melbourne, Australia; [5]Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Recent rodent studies suggest that the claustrum complex, an evolutionarily conserved structure with widespread cortical connectivity, modulates anxiety-like behaviour via projections to the basolateral amygdala. However, this circuitry remains poorly defined in primates. Here, we investigated structural connectivity between the claustrum complex, amygdala, and prefrontal cortex in the adult common marmoset (Callithrix jacchus) using diffusion-weighted tractography (n = 30; 22 males, 8 females) and neuroanatomical tracing (n = 5; 4 males, 1 female). Tracer injections were performed under anaesthesia via stereotaxic surgery. One marmoset received an injection of biotinylated dextran amine (BDA) into the basolateral amygdala, a tracer with anterograde and retrograde transport properties. Four others received fluorescent retrograde tracers targeting the frontopolar cortex (area 10), orbitofrontal cortex (area 11), medial prefrontal cortex (area 32V), and primary somatosensory cortex (area 3b). After a 10-15-day survival period, brains were perfused, cryoprotected, sectioned at 40 μm, and processed for fluorescence microscopy, Nissl and Myelin staining, and BDA histochemistry. Labelled neurons and axons were mapped onto cytoarchitecturally defined claustrum subdivisions to identify monosynaptic connections. Diffusion-weighted imaging was conducted on a 9.4 T Bruker MRI using a 40 mm volume resonator (81 directions, b = 5000 s/mm², 125 μm isotropic resolution, ~18-hour scan time). Data were preprocessed and modelled using multi-shell, multi-tissue constrained spherical deconvolution in MRtrix3. Regions of interest—including amygdala subnuclei, orbitofrontal cortex (areas 11 and 13), medial prefrontal cortex (areas 32V and 25), claustrum complex, and area 3b—were segmented in ITK-SNAP and coregistered to diffusion space for tractography-based region-of-interest analysis. We found that the dorsal endopiriform nucleus was the region of the claustrum complex with the highest structural connectivity with both the amygdala and prefrontal cortex, showing particularly strong connectivity with the lateral amygdala and posterior orbitofrontal cortex, and more moderate connectivity with the medial prefrontal cortex. These findings delineate a distinct claustro-amygdalo-frontal subcircuit and provide the first detailed topographic map of this pathway in the non-human primate, highlighting the dorsal endopiriform nucleus as a potential target for future anxiety-related circuit research.

Disclosures: B. Hoang: None. **D. Wright:** None. **A.C. Roberts:** None. **M.G. Rosa:** None. **D.H. Reser:** None.

Poster

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Program #/Poster #: PSTR060.22/C43

Topic: B.07. Network Interactions

Support: CIHR Grant 192040

Title: Claustro-cingulate and claustro-prelimbic projections differentially modulate nociception

Authors: *C. A. FAIG[1,2], J. LINDNER[2], J. JACKSON[3,2], A. M. W. TAYLOR[1,2,4];
[1]Pharmacol., [2]Neurosci. and Mental Hlth. Inst., [3]Physiol., [4]Oncology, Univ. of Alberta, Edmonton, AB, Canada

Abstract: The prelimbic (PrL) and anterior cingulate (ACC) cortices are critical in controlling pain sensitivity by facilitating or inhibiting descending pain modulation. The PrL, which is anti-nociceptive, loses its excitatory inputs in chronic pain leading to suppression. Meanwhile the ACC, which is pro-nociceptive, becomes hyperexcitable due to a loss of inhibition. Together, this drives pain hypersensitivity. Unfortunately, the source of inhibitory drive in these structures is not well understood, making it difficult to resolve this hypersensitivity. Interestingly, projections from the claustrum can engage feedforward inhibition of the cortex and is densely connected to both the PrL and ACC. Our previous work identified claustro-cingulate projections that were activated by acute nociception but become selectively inactivated in chronic pain. Inhibiting this pathway induced pain hypersensitivity, suggesting that they normally inhibit the ACC to restrict pain processing. Using a retro-AAV encoding Cre recombinase into the ACC or PrL and an AAV encoding excitatory (hM3Dq) or inhibitory (hM4Di) designer receptors we compared the role of claustro-cingulate and claustro-prelimbic projections in pain processing. Baseline mechanical thresholds of young male c57BL6j mice were acquired after an injection of vehicle control or designer receptor agonist deschloroclozapine. Then mice received a hind paw injection of complete Freund's adjuvant to induce chronic pain. Following excitation of the claustro-prelimbic projections, mice displayed mechanical hypersensitivity similar to claustro-cingulate inhibition. Interestingly, neither claustro-cingulate inhibition nor claustro-prelimbic excitation induced aversive associative memories in a conditioned place aversion test despite enhancing baseline pain sensitivity. After pain hypersensitivity resolved, claustro-prelimbic activation was still able to induce significant hypersensitivity while claustro-cingulate inhibition couldn't, supporting previous findings that suggested a selective dysfunction in claustro-cingulate projections. Conversely, claustro-prelimbic inhibition had no effect on baseline sensitivity but did significantly rescue chronic pain induced hypersensitivity similar to previous claustro-cingulate activation experiments. Together, these results suggest that claustrum projection populations can differentially modulate pain sensitivity by targeted inhibition of the PrL or the ACC. Furthermore, dysfunction of claustro-cingulate projections but not claustro-prelimbic projections may explain the selective loss of ACC inhibition in chronic pain.

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Poster

PSTR060: Network Interactions

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Topic: B.07. Network Interactions

Support: R.B. Stein Physiology Graduate Scholarship
Canadian Institutes of Health Research

Title: Investigation into the activity of claustrum interneurons across different brain states

Authors: *A. D. DO, J. C. JACKSON;
Physiol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Cortical networks constantly fluctuate between distinct activity patterns reflecting changes in brain state. During low arousal states, such as rest and 'reflexive' behaviours, a synchronized state arises with a coordinated increase in low-frequency oscillations (<4Hz) in cortical regions. By contrast, movement and high arousal are accompanied by a desynchronized state and a predominance of cortical gamma rhythms (30-100Hz). The claustrum is a small subcortical brain region densely and reciprocally connected with the entire cortex. Recent experiments in rodents, reptiles, and humans have highlighted the state-dependent activity pattern of claustrum neurons projecting to the cortex. Claustrocortical neurons are most active during synchronized states and are mostly suppressed during desynchronized states. The underlying neuronal circuits for this state-dependent modulation of the activity of claustrum projection neurons is unknown. Inhibitory regulation of claustrocortical neurons by local GABAergic interneurons may permit a precisely timed engagement or disengagement of claustrum outputs according to changes in brain state. We use fiber photometry in behaving mice to investigate the in vivo contribution of local interneurons in the claustrum in shaping the activity of claustrum projection neurons during cortical synchronization and desynchronization. A better understanding of how the activity of different populations of claustrum neurons relates to brain states is essential in the investigation of the function of the claustrum.

Disclosures: A.D. Do: None. **J.C. Jackson:** None.

Poster

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Topic: B.07. Network Interactions

Support: 25-BR-02-02
25-BR-07-01
NRF-2023-00248148

Title: Molecular and morphological characterization of Layer 6b and claustrum neurons in the neocortex

Authors: *J. LEE[1,2], S.-J. KIM[3], K. LEE[4], S. P. BROWN[5], J. KIM[4,6];
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Abstract: To understand neocortical function, it is helpful to first define its constituent cell types. Recent studies indicate that neurons in the deepest cortical layer play roles in mediating thalamocortical interactions, modulating brain state, and are implicated in neuropsychiatric diseases. However, understanding the functions of deep layer 6 (L6b) neurons has been hampered by the lack of a consensus definition for these cell types. In this study, we identified L6b neurons using the molecular markers Complexin 3 (Cplx3) and Connective Tissue Growth Factor (CTGF), along with the retrograde tracer Cholera Toxin Subunit B (CTB). By tracing neurons in the sensory and motor cortices of both hemispheres, we observed that L6b neurons send axons to upper layer of the same (ipsilateral) cortex, but not to the opposite (contralateral) hemisphere. Morphologically, these cells are multipolar with spiny dendrites, and their processes remain within the infragranular layers. Retrograde tracing from the thalamus, combined with CTGF labeling, showed little overlap, suggesting that L6b neurons do not project to the thalamus. To determine whether these neurons are excitatory or inhibitory, we examined co-expression of CTGF with parvalbumin (PV) and somatostatin (SOM), markers for major inhibitory interneurons. The lack of overlap indicates that CTGF(+) L6b neurons are excitatory. Since L6b and claustrum share similarities in their development, location, and potential functions, we also study the molecular characteristics of the claustrum neurons with CTGF and Cplx3. These studies provide molecular and anatomical criteria for identifying L6b and claustrum neurons and set the stage for future research into their specific functions in cortical and brain-wide neuronal circuitry.

Disclosures: J. Lee: None. **S. Kim:** None. **K. Lee:** None. **S.P. Brown:** None. **J. Kim:** None.

Poster

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Program #/Poster #: PSTR060.25/C46

Topic: B.07. Network Interactions

Support: KBRI basic research program through Korea Brain Research Institute funded by Ministry of Science and ICT (25-BR-02-02)

Title: Cell type-specific analysis of psilocybin effects on the mouse somatosensory cortex and claustrum

Authors: *M. KANG[1], J. LEE[2,3], J. KIM[4];
[1]Emotion, Cognition & Behavior Group, Korea Brain Res. Institute, Daegu, Republic of Korea,, daegu, Korea, Republic of; [2]Korea Brain Res. Inst., Daegu, Korea, Republic of; [3]Sch. of Med., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [4]Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of

Abstract: Psychedelics, a subclass of hallucinogens, modulate the central nervous system, eliciting sensory experiences such as auditory and visual hallucinations, and enhancing euphoria and self-awareness. Recent research suggests that psychedelics like psilocybin, MDMA, and LSD hold promise as neurotherapeutics for psychiatric disorders, though their underlying neural mechanisms remain unclear. A recent fMRI study in animal models and human indicated that psilocybin alters the activity of the cortical regions and claustrum. In this study, we hypothesize that psilocybin alters neuronal activity in the cortex and claustrum in a cell type-specific manner. To investigate the effects of psilocybin on the regions, we analyzed c-Fos expression in a region- and layer-specific manner across cortical areas after psilocybin treatment. Our results revealed increased c-Fos(+) cell density in most cortical areas and claustrum. Based on these findings, we further examined the electrophysiological properties of cortical and claustral neurons in the somatosensory cortex. Using whole-cell patch-clamp recordings, we assess the effects of the psychedelic drug on basic membrane properties and intrinsic excitability of the neurons.. This study provides biological evidences how psilocybin modulates neuronal activity in a cell type-, region- and layer-specific manner.

Disclosures: M. kang: None. **J. Lee:** None. **J. Kim:** None.

Poster

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Topic: I.04. Executive Functions

Support: MH118500

Title: Topographical core and matrix innervation of the thalamic reticular nucleus in human and non-human primates.

Authors: *J. SON[1], X. LIU[1], N. MATUK[1], H. BARBAS[2], B. ZIKOPOULOS[1];
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Abstract: The thalamus can redirect, initiate, sustain, and switch cortical activity. Thalamocortical (TC) pathways can be divided by structural circuit motifs into core and matrix. In primates, excitatory core TC pathways include parvalbumin-positive thalamic projections that target mainly the middle cortical layers, drive cortical activity, and are involved in sensory, motor, and cognitive processes. Excitatory matrix TC pathways originate from calbindin-positive thalamic projection neurons that project mainly to the upper cortical layers and carry high-order signals from the internal environment. In addition, the thalamic reticular nucleus (TRN), an inhibitory nuclear net that encases the thalamus, can intercept TC pathways and filter incoming signals to the cortex. Due to its strategic position between thalamus and cortex, the TRN receives topographically organized collateral projections from both structures, organized into rostrocaudal sectors for distinct modalities. Here, we describe the organization and relative proportion of immunohistochemically labeled core and matrix TC pathways traveling through and forming synapses at different regions of the rhesus macaque and human TRN. Our study uncovered a heterogeneous pattern of calcium-binding protein expression in TC pathways that systematically varied along the dorsoventral extent of TRN and the adjacent white matter tracts in the internal capsule. Core innervation was prevalent in ventral TRN, where the ratio of core/matrix axons and synapses was high but dropped rapidly up the dorsal axis, where it was almost halved. Matrix innervation was distributed in an inverse, yet gradual gradient with a higher density dorsally. Comparison revealed similarities in core and matrix innervation patterns across the human and non-human primate TRN. Our findings suggest that core and matrix TC projections target the TRN in a topographical manner and are organized in a dorsoventral pattern. This dorsoventral pattern extends across anatomical and functional sectors that are organized along the rostrocaudal axis, revealing a novel segmentation of TRN into broad core and matrix regions that are defined by functional, connectional, and molecular features. This layer of TRN organization in primates likely sets the stage for rhythmic brain activity, the sleep/wake cycle, memory consolidation, states of vigilance, attention, and sensory processing, and may be differentially affected in disorders, such as schizophrenia and autism.

Disclosures: J. Son: None. **X. Liu:** None. **N. Matuk:** None. **H. Barbas:** None. **B. Zikopoulos:** None.

Poster

PSTR060: Network Interactions

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR060.27/C48

Topic: B.07. Network Interactions

Title: Continuous and Intermitted Deep Brain Stimulation of Subthalamic and Substantia Nigra Pars Reticulata modulates Pedunculopontine

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Abstract: Even though the underlying mechanism is still unknown, current clinical treatment results show intermittent DBS (iDBS) can generate similar results to continuous DBS (cDBS) on some pediatric patients with dystonia based on standard clinic movement tests. Previous studies show that intracranial signal recording from certain brain area such as STN and PPN for the bandwidth below 50Hz is real-time correlate of motor symptom severity for Parkinson disease and has strong correlation with movement for pediatric dystonia disease. Our hypothesis is that the function of iDBS is different to the function of cDBS. Therefore, analyzing the power spectral density below 50Hz of intracranial signal recordings in brain regions such as STN/SNr and pedunculuspontus nucleus (PPN) is one way to study the differences between iDBS and cDBS.Existing studies have shown that subthalamic (STN) deep brain stimulation (DBS) is an effective treatment method for alleviating motor symptoms. Pedunculopontine nucleus (PPN) has been studied as a target for DBS, mainly for adult patients with dystonia or movement disorders in Parkinson's disease, showing improvements in postural instability and gait dysfunction. Because it is difficult to target PPN using standard techniques, it is usually not selected as a target for adult or pediatric pathology. The STN directly innervates the PPN, and also indirectly through collaterals of the STN to basal ganglia (GPi) and STN to substantia nigra pars reticulata (SNr) pathways to form a bidirectional circuit, which is involved in motor control and locomotion.During the Neuromodulation Monitoring Unit (NMU), we temporarily implant AdTech MM16C depth leads into multiple targets, including GPi, STN/SNr, Vo, VIM and PPN, which allows us to deliver stimulation by macro-contacts and simultaneously record the local brain signals from micro-contacts. Based on the patients' tolerance, we set 1.5V or 3V for continuous DBS (cDBS) and stimulate at 55Hz in the SNr and STN, while intermitted DBS (iDBS) was set to be approximately cycled on/off for 100ms, which one cycle is around 200ms. The frequency and voltage settings for iDBS are set according to the setting of each patient's cDBS.

Disclosures: Y. Sun: None. **M. Kasiri:** None. **A. Seyyed Mousavi:** None. **T.D. Sanger:** None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.01/C49

Topic: B.08. Epilepsy

Support: ICMR Grant

Title: Crosstalk Between Platelets and Neural Cells in Drug-Resistant Epilepsy: Mechanisms Driving Hyperexcitability

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Abstract: Purpose: Drug-resistant epilepsy (DRE) is characterized by neuronal hyperexcitability, largely driven by an imbalance between excitatory and inhibitory neurotransmission. While much attention given to neural cells, emerging evidence highlights a role for platelets in modulating neural excitability. Platelets, act as reservoirs and releasers of an array of bioactive molecules, including neurotransmitters. Recent studies suggest that platelet degranulation can be triggered by specific miRNAs. In the context of epilepsy, BBB disruption permits platelet infiltration into the brain parenchyma, where these cells may crosstalk with neural elements influencing synaptic activity. However, the precise contribution of platelet-derived molecules to neuronal hyperexcitability and seizure generation remains poorly understood. Here we investigated the platelet-mediated neurotransmitter release in both platelets and brain tissues from DRE patients, levels of miRNA-223 which is involved in activation of platelets. We also assessed receptor expression in brain tissue to elucidate how platelet-derived neurotransmitters may interact with specific neural receptors, thereby altering excitatory and inhibitory signaling pathways as observed in DRE. **Method**: Blood and brain tissue samples were collected from DRE patients (n=13). Age and region matched postmortem brain samples (control) were collected as control (n = 7). Serotonin, histamine and GABA levels and GABA - Transaminase were measured in serum, platelet pellets, and brain tissue using ELISA and HPLC. Histamine receptor expression was observed using q-PCR. Comparisons between groups were performed using Mann-Whitney U Test. **Results:** Tissue samples resected from DRE patients showed a significant increase in serotonin levels compared to autopsy controls ($p < 0.05$). Similarly, serotonin levels were significantly elevated in the serum of DRE patients, with a corresponding trend observed in platelets ($p < 0.05$). Additionally, GABA-T activity was found to be significantly higher in both tissue and platelet samples from DRE patients. Moreover, miRNA-223 was upregulated in DRE patients as compared to control ($p < 0.05$). **Conclusion:** Elevated serotonin levels in serum and platelets of DRE patients suggests that abnormal serotonin release by platelets contributes to the disruption of neurotransmitter balance. Moreover, upregulated miRNA-223 in DRE patients indicates increase in platelet activation. This study underscores the potential of targeting platelet-mediated processes to restore neurotransmitter balance and reduce seizure activity in DRE patients.

Disclosures: A. Sharma: None. **J. Banerjee:** None. **A. Dixit:** None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.02/C50

Topic: B.08. Epilepsy

Support: KHIDI Grant

Title: Somatic RAS-activating mutations in the neurogenic niche and genotype-phenotype correlations in mesial temporal lobe epilepsy with hippocampal sclerosis

Authors: *J. KIM[1,2], S. LEE[3,2], S. KIM[2];
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Abstract: Purpose:The genetic basis of mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) remains poorly understood, particularly with respect to the unique roles of hippocampal subregions such as the dentate gyrus (DG) and cornus ammonis (CA). This study aimed to uncover somatic variants within the neurogenic niche that may contribute to MTLE pathogenesis. **Method**:Hippocampal tissues were obtained from 40 MTLE patients who underwent surgical resection, including 34 with matched peripheral blood and 6 without. DNA was extracted using laser capture microdissection from the DG, CA, and subiculum. Due to limited tissue amounts, we utilized low-input DNA sequencing with enzymatic fragmentation. Deep whole-exome sequencing was performed, achieving ~500x coverage in both brain and blood samples. Somatic variants were detected using Mutect2, followed by filtering with FilterMutectCalls, MosaicForecast, and SOBDetector to minimize artifacts from formalin-fixed paraffin-embedded samples. **Results**:The majority of somatic variants were cytosine-to-thymine transitions, associated with SBS (single base substitution) signatures 1, 5, 16, and the hypermutation-related SBS10b, the latter emerging as a novel signature in epilepsy. RAS pathway-activating mutations were detected in 8 patients, including gain-of-function mutations in oncogenes (e.g., BRAF, CAMK2B) and loss-of-function mutations in tumor suppressors (e.g., NF1, DAB2IP). Moreover, loss-of-function mutations in TP53 were identified in three patients, all of whom also harbored co-occurring driver mutations in cancer-related genes. These mutations were more commonly found in the DG than in the CA, and SBS1 and SBS10b were notably enriched in RAS-mutant samples. Clinically, RAS-positive patients demonstrated a hypermutated profile, granule cell dispersion, earlier seizure onset, and an absence of febrile seizure history; however, surgical outcomes did not differ significantly between groups. **Conclusion**:This study highlights the role of RAS-activating somatic mutations in the DG as potential drivers of epileptogenesis in MTLE-HS. The distinct genomic and clinical features observed in RAS-positive individuals support the contribution of somatic mosaicism to the development of this disease.

Disclosures: J. Kim: None. **S. Lee:** None. **S. Kim:** None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.03/C51

Topic: B.08. Epilepsy

Support: National Center for Advancing Translational Sciences, National Institutes of Health UL1 (# TR001860)
UC Davis Clinical Translational Science Center (CTSC) KL2 Award (# TR001859)

Title: Distinguishing Seizure Types Using Spectral Coherence

Authors: *K. KIVIAT[1], Z. MCNAUGHTON[2], J. PATEL[2], C. ARMSTRONG[1], S. TOPRANI[1]; [1]Neurol., [2]Univ. of California, Davis, Davis, CA

Abstract: Epilepsy patients can have multiple types of seizures, originating from different locations and following different spread patterns. It is important to identify these distinct types to understand where intervention may be required to treat the seizures. While these seizure types can be determined by visually inspecting intracranial EEG (iEEG) signals, it is useful to (1) computationally characterize seizure types (e.g., by effective connectivity) to determine whether visual differences reflect quantifiably distinct seizures versus different parts of a spread pattern, (2) characterize future seizures to determine if they are the same as those already characterized or a new type, and (3) compare stimulated seizures to each typical seizure type or distinguish atypical/aberrant stimulated seizures. It is important for clinical decision-making to know whether a stimulated seizure is representative of typical spontaneous seizure to determine where to focus surgical treatment.

We have characterized continuous iEEG data over 1-3 weeks during seizures (3-60 per patient) by calculating spectral coherence in a series of time windows between all pairs of recording electrodes in a range of physiological frequency bands to estimate effective connectivity across the brain network in patients with refractory epilepsy undergoing surgical planning. These pairwise coherence values define a location in a high-dimensional state space for each time window. Using the dimension reduction algorithm PaCMAP (Pairwise Controlled Manifold Approximation), we can visualize this high-dimensional space in low dimensions while preserving important structure. This separation is made more rigorous by reducing the dimensionality of the data through principal component analysis and applying logistic regression to classify seizure types.

In patients (n=94 seizures across 2 patients) with multiple seizure types, PaCMAP analysis shows different seizure types forming separate effective connectivity clusters, while in a patient with only one seizure type (n=25 seizures from 1 patient), the seizures do not separate. Correspondingly, the distinct seizure types can be successfully classified using logistic regression. In a patient with stimulated seizures, both PaCMAP visualization and logistic regression showed that the stimulated seizures could not be distinguished from spontaneous seizures. This is consistent with the clinical evaluation that the stimulated seizures were typical. Thus, spectral coherence applied to iEEG contacts defines a state space that can separate distinct seizure types and may be able to distinguish atypical stimulated seizures from typical ones.

Disclosures: K. Kiviat: None. **Z. McNaughton:** None. **J. Patel:** None. **C. Armstrong:** None. **S. Toprani:** None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.04/C52

Topic: B.08. Epilepsy

Title: In vitro mechanism of epileptogenesis in focal cortical dysplasia pediatric brain tissue containing high frequency oscillations

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Abstract: Introduction: Focal cortical dysplasia (FCD) is a developmental brain abnormality caused by disrupted neuronal migration and organization, often leading to drug-resistant epilepsy in pediatric patients. In such cases, localizing and fully resecting the epileptogenic zone (EZ) is critical for achieving postoperative seizure freedom. The EZ contains abnormal neuronal networks responsible for seizure generation, though their exact mechanisms remain poorly understood. This study investigates synaptic properties within resected EZ tissue to better understand seizure initiation and pharmacoresistant epilepsy in pediatric patients with FCD. **Methods**: Pediatric patients with FCD and refractory epilepsy underwent pre-surgical localization of the EZ using stereoelectroencephalography (sEEG). Brain specimens containing high-frequency oscillations (HFOs) and adjacent cortical tissue were resected separately. Tissue was sliced into 400 μm horizontal sections using a vibratome. Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded using field potential and single-cell patch-clamp techniques. Additionally, inhibitory currents and interictal epileptiform discharges (IEDs) were analyzed after bath application of pro-convulsants 4-aminopyridine and bicuculline. **Results:** sEPSCs recorded from EZ tissue with HFOs showed significantly shorter intervals, increased amplitude, and prolonged duration compared to adjacent cortex without HFOs. Following treatment with 4-aminopyridine and bicuculline, no difference was found in GABAb-mediated outward currents between tissue types. However, IEDs occurred more frequently in EZ tissue with HFOs, indicating increased excitability. **Conclusion:** EZ tissue containing HFOs in FCD exhibits heightened excitatory input and a greater propensity for seizure generation. Despite similar inhibitory GABAb responses across tissues, the elevated excitatory activity and increased susceptibility to pro-convulsants suggest a disrupted excitation-inhibition balance. This imbalance likely contributes to pharmacoresistant epilepsy in pediatric FCD patients. These findings provide insight into the epileptogenic mechanisms of FCD and may inform future therapeutic strategies targeting network hyperexcitability in affected children.

Disclosures: A. Cattani: None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.05/C53

Topic: B.08. Epilepsy

Support: JSPS KAKENHI Grant Number 24K10971

Title: Epilepsy surgery outcomes by using the onset of interictal spike-related ripples

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Abstract: Accurate identification and complete resection of the epileptogenic zone (EZ) are fundamental requirements for achieving seizure freedom in patients undergoing epilepsy surgery. Although ictal electrocorticography (ECoG) is widely used to guide EZ localization, surgical resections based solely on ictal findings result in seizure-free outcomes in only approximately 60% of cases. Therefore, more reliable interictal biomarkers are needed to enhance surgical precision. High-frequency oscillations (HFOs), especially ripples within the 80-200 Hz frequency range, have emerged as potential indicators of epileptogenicity. Previous studies have suggested that ripple activity correlates with the EZ, but the significance of ripple onset in relation to interictal spikes has not been fully explored. We hypothesized that identifying the onset channels of ripples temporally linked to interictal spikes could improve EZ localization and enhance postsurgical outcomes. To investigate this hypothesis, we developed a method using complex demodulation to detect the precise timing and location of ripple onset associated with interictal spikes. We retrospectively analyzed 12 patients who underwent focal cortical resection and became seizure-free postoperatively. In all cases, ripple onset channels were included within the resected area. Specifically, 77% of the electrodes at 5 ms after ripple onset, 60% at 10 ms, and 58% at 15 ms were within the resection margin. Subsequently, we applied this approach prospectively in 39 patients undergoing epilepsy surgery. Among those whose ripple onset channels were resected, 73.3% achieved seizure freedom, while 66.7% of patients in whom the ripple onset was not included in the resection continued to experience seizures. Statistically, ripple onset resection was found to be associated with seizure resolution ($p=0.048$, Fisher's exact test). These results indicate that early onset of spike-related ripples in the interictal period is a reliable and clinically useful marker of EZ. Our findings suggest that incorporating ripple onset channels into the surgical planning process has the potential to significantly improve the precision of EZ localization and overall surgical outcomes in patients with drug-resistant epilepsy. This method is simple, reproducible, and may be implemented as a complementary tool alongside conventional ECoG interpretation.

Disclosures: M. Uematsu: None. **Y. Numata-Uematsu:** None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.06/C54

Topic: B.08. Epilepsy

Support: Funding was provided through the NIH Intramural Research Program

Title: Interictal MEG reveals deviations from critical dynamics and altered excitation-inhibition balance in patients with focal epilepsy

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Abstract: Healthy brains maintain a delicate balance between excitation (E) and inhibition (I), exhibiting critical dynamics characterized by spatiotemporal cascades of activity known as neuronal avalanches (NAs). In epilepsy, increased excitation predominates during seizures. However, E/I alterations remain less clear in the interictal state (between seizures). Here, we leverage resting-state interictal magnetoencephalography (MEG) recordings to develop NA-based biomarkers to assess interictal E/I balance in patients with drug-resistant focal epilepsy. We hypothesize that inhibition predominates during the interictal state, suppressing excitation and thereby seizures, resulting in a sub-critical brain state with suppressed propagation of NAs compared to healthy controls. We acquired six-minute resting state interictal MEG recordings free of overt epileptiform activity from 55 patients (32±10 years, 22 female) with drug-resistant focal epilepsy undergoing pre-surgical evaluation at the NIH Clinical Center, and 55 healthy controls (33±10 years, 31 female). NAs were detected as spatiotemporal cascades of discrete events extracted from large deflections in the z-scored MEG signals across channels. NAs were characterized using: 1) the branching parameter (σ) computed as the ratio of events across consecutive time steps, where $\sigma=1$ reflects critical, balanced E/I dynamics, and $\sigma<1$ indicates a sub-critical, inhibition-dominant state; 2) number of NAs per minute; and 3) total time in NAs (avalanche lifetime). Statistical analysis was performed using the Mann-Whitney U test. Predictive performance was assessed using area under the receiver-operating characteristic curve (AUC) and odds ratio (OR) with 95% confidence intervals. Compared to healthy controls, epilepsy patients showed significantly lower σ values ($\sigma<1$; p=0.02, AUC=0.67, OR=0.6 (0.4-0.8)), fewer NAs per minute (p=4.12e-7, AUC=0.81, OR=0.3 (0.2-0.5)), and shorter avalanche lifetimes (p=4.61e-7, AUC=0.79, OR=0.3 (0.2-0.5)). The OR values <1 for all biomarkers indicate they were significant ($p<0.05$) negative predictor of epilepsy. Our findings suggest that during interictal periods, even in the absence of overt epileptiform activity, epilepsy patients exhibit reduced prevalence and propagation of NAs, reflecting an inhibition-dominant, sub-critical brain state. Ongoing work aims to localize these effects to support identification of epileptogenic zones for pre-surgical evaluation.

Disclosures: P. Eedara: None. **A.I. Triggiani:** None. **Y. Serlin:** None. **J. Stout:** None. **W.H. Theodore:** None. **S. Inati:** None.

Poster

PSTR061: Human Epilepsy

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Program #/Poster #: PSTR061.07/C55

Topic: B.08. Epilepsy

Support: CIFRE 2023/0269

Title: Fast Ripples Through Different Lenses: The Scale Effect of Micro- and Macro- Electrode Recordings in Human Patients with Epilepsy

Authors: *B. MARCY[1], L. VALTON[2], A. DE BARROS[3], M. DENUELLE[3], E. J. BARBEAU[4], J. CUROT[5];
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Abstract: The clinical deployment of deep hybrid micro-macroelectrodes has enabled the recording of electrophysiological events on a micro-scale, which were previously observable only on a macro-scale. This study investigates whether Fast Ripples (FR), high-frequency oscillations (200-600 Hz) that serve as biomarkers for epileptogenic tissue with highly focal generators (≈0.8 mm³), exhibit the same electrophysiological profile when recorded on micro- and macro-scales. We analyzed data from 40 patients undergoing stereo-electroencephalography at Toulouse University Hospital using hybrid DIXI Medical electrodes (macrocontacts, tetrodes combining 4 microelectrodes, as part of the EPIFAR (NCT02491476) and STIMIC (NCT03738072) clinical trials. Recordings were acquired using Blackrock (n=31) and Neuralynx (n=9) systems, with FRs detected over a one-hour period per patient using Halyzia software. Metrics such as entropy, frequency, FR-index (Ratio of energy range distribution over the frequency range, indicator of the pathogenicity), duration, amplitude, and timing of occurrence were evaluated. FRs have been classified into two FR ranges, low-frequency FRs from 200 to 300 Hz and high-frequency FRs from 300-600 Hz. FRs vary between micro- and macro-scales. Tetrodes exhibit a higher yield in high-frequency FRs. Micro-FRs (0.21 +- 0.10) show a significant difference from macro-FRs (0.16 ± 0.08) in the FR-index ($p = 0.027$). Within micro-FRs, high-frequency FRs (0.25 +- 0.10) differ from low-frequency FRs (0.17 +- 0.09) in the FR-index ($p = 0.015$). There is a tendency for high-frequency macro- and micro-FRs to differ in entropy (0.78 +- 0.04 vs. 0.72 +- 0.11; $p = 0.053$). Additionally, it appears that most FRs recorded simultaneously at both scales are not entirely synchronous, a phenomenon we are currently quantifying. These findings suggest that FR populations captured at the microelectrode scale differ from those at the macroelectrode scale, with the micro-scale likely better capturing the most pathogenic FRs. These results highlight the importance of considering scale in clinical interpretations.

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Poster

PSTR061: Human Epilepsy

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Program #/Poster #: PSTR061.08/C56

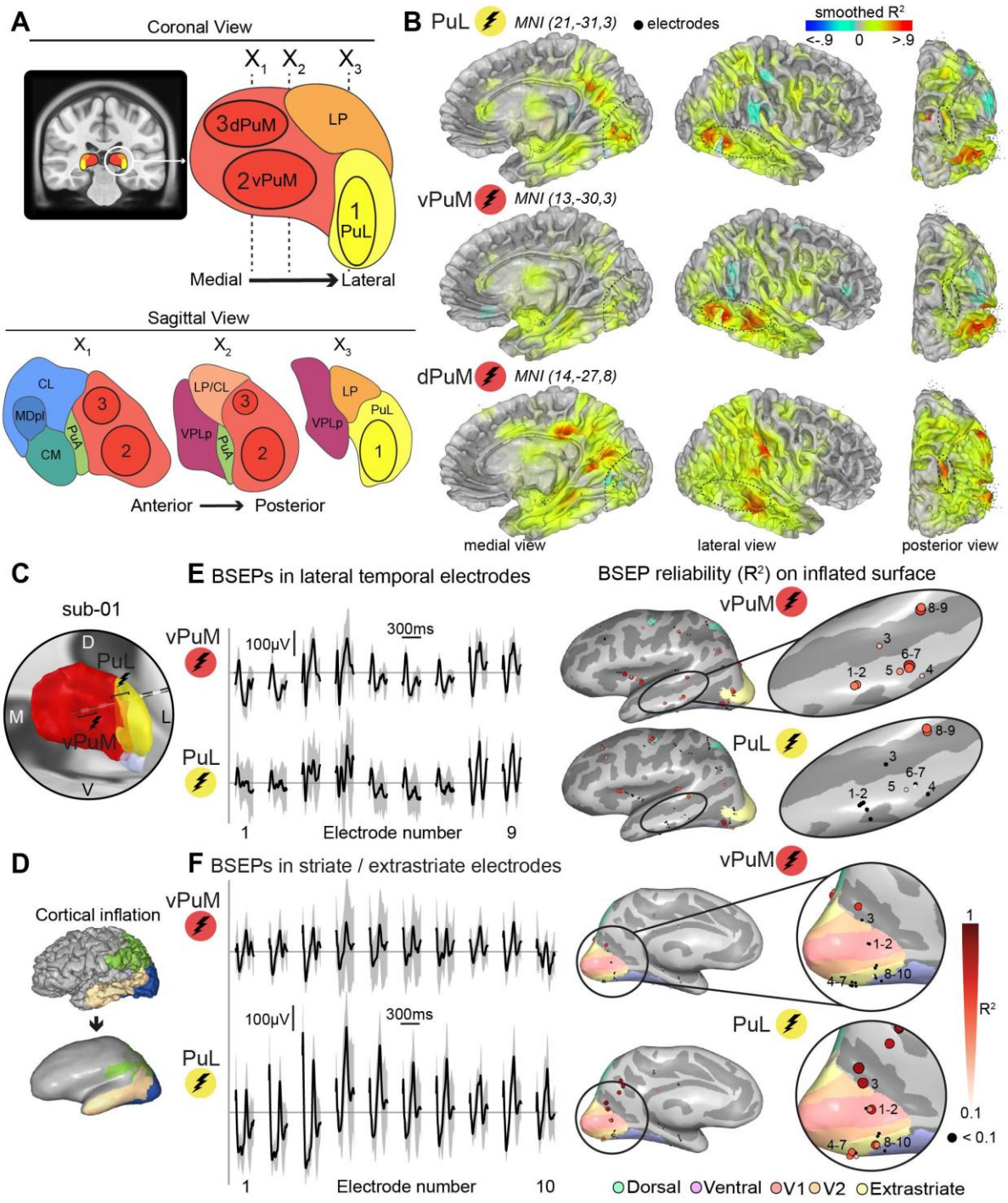
Topic: B.08. Epilepsy

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P41-EB018783
U01-NS128612

Title: Human pulvinar stimulation engages select cortical pathways in epilepsy

Authors: *J. A. BILDERBEEK[1], N. GREGG[2], M. YÁÑEZ-RAMOS[1], H. HUANG[1], M. MONTOYA[1], P. BRUNNER[5], J. T. WILLIE[6], J. J. VAN GOMPEL[3], G. A. WORRELL[4], K. J. MILLER[3], D. HERMES[1];
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Abstract: The pulvinar has been proposed as an effective neuromodulation target for patients with posterior quadrant and temporal epilepsies. However, the pulvinar has a large tissue volume, multiple subnuclei, and widespread cortical connections. It remains unknown whether electrical stimulation of distinct pulvinar subregions affects the temporal, occipital, and parietal areas differently. To address this gap, we delivered single-pulse electrical stimulation to the pulvinar and measured the resulting brain stimulation evoked potentials in twelve patients undergoing stereotactic EEG for drug-resistant epilepsy. Brain stimulation evoked potentials were parameterized across the occipital, temporal and parietal cortex. Stimulation of the lateral pulvinar elicited significant brain stimulation evoked potentials in striate and extrastriate areas that diminish as stimulation shifts towards the medial pulvinar. Conversely, stimulation of the ventral aspect of the medial pulvinar produced significant lateral temporal evoked potentials, which diminish with lateral pulvinar stimulation. We also found that stimulation of the dorsomedial pulvinar evoked significant parietal responses with limited striate/extrastriate and lateral temporal responses. These results demonstrate that electrical stimulation of specific pulvinar subregions influences distinct occipital, parietal and lateral temporal areas. Selective targeting of pulvinar subregions to maximize seizure network engagement may be essential for individualized treatment of posterior quadrant and temporal epilepsies.



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Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience, NeuroOne. F. Consulting Fees (e.g., advisory boards); NeuroOne. Other; Investigator for SLATE Trial, Investigator for Medtronic EPAS Trial, Mayo Clinic Medtronic UH3-NS95495, Investigator for NXDC Gleolan Men301 Trial, Investigator for MRgUS EP001 Trial, Investigator for Polyganics ENCASE II Trial. **G.A. Worrell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience, NeuroOne. F. Consulting Fees (e.g., advisory boards); NeuroOne, UNEEG, LivaNova, NeuroPace. **K.J. Miller:** None. **D. Hermes:** None.

Poster

PSTR061: Human Epilepsy

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Program #/Poster #: PSTR061.09/C57

Topic: B.08. Epilepsy

Support: Jordan Elizabeth Harris Foundation

Title: Adolescents with epilepsy show reduced effective connectivity dynamics during emotional conflict: A MEG study

Authors: *F. KING[1,2], M. PERRY[2], C. PAPADELIS[2,3], C. M. COOPER[4,2];
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Abstract: Emerging evidence suggests that epilepsy arises from preexisting brain network dysfunction. However, there is a need to investigate network functioning in young and newly diagnosed epilepsy patients to assess whether connectivity deficits exist prior to long-term seizure exposure. Most research relies on resting-state fMRI, highlighting the need for high-temporal-resolution methods to examine effective connectivity during cognitively demanding tasks. This study uses magnetoencephalography (MEG) to examine task-based effective connectivity during emotional conflict processing in adolescents with epilepsy. Twenty-eight adolescents with epilepsy and 25 controls (27 females; ages 10-20, mean age = 15) completed an Emotional Face-Word Stroop task during MEG recording. Effective connectivity was assessed using Granger causality, and graph theory metrics—including global efficiency, path length, and node-level strength, degree, and betweenness—were computed for nodes in the Default Mode, Salience, Frontoparietal Control, and Somatomotor networks across nine overlapping 200 ms windows from 1-1000 ms post-stimulus. Group differences were analyzed using MANOVAs for each time window (Bonferroni corrected), followed by post-hoc FDR-corrected t-tests at the node level. Controls showed dynamic, task-appropriate changes in connectivity over time, whereas the epilepsy group exhibited relatively static patterns. In controls, early (~0-0.5s) effective connectivity centrality peaked in Default Mode and Salience Network regions, significantly exceeding that of the epilepsy group between 0.2-0.4s (ps < .05). This early peak

declined over time in controls, while the epilepsy group maintained a static level, leading to late (~0.5-0.7s) hyperconnectivity in a Salience Network node ($p < .05$). As expected, controls displayed low early connectivity in the Frontoparietal Control network that increased later (~0.5-1.0s), while adolescents with epilepsy showed static connectivity resulting in significantly higher early centrality (0.2-0.4s, ps $< .05$). Additionally, the epilepsy group exhibited significantly lower global efficiency and higher path length (lambda) during the earliest window (0-0.2s, ps $< .05$), indicating disrupted large-scale network integration. This study reveals reduced dynamic connectivity and early network efficiency in adolescents with epilepsy, suggesting network dysfunction may precede seizure-related cognitive decline. These deficits may underlie common psychiatric comorbidities and could help improve diagnosis and treatment if shown to vary by seizure type or predict treatment response.

Disclosures: F. King: None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.10/Web Only

Topic: B.08. Epilepsy

Support: NIH K99-R00: K99NS138680

Title: Automated harmonization of intracranial EEG signals to mitigate site-specific batch effects in epilepsy

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Abstract: Inter-site variability in intracranial EEG (iEEG) hinders development of generalizable epilepsy biomarkers for normative multi-cohort modeling. We investigated batch effects across 5,847 electrode recordings from two centers (Penn: n=45, 4,075 total electrodes; MNI: n=106, 1,772 total electrodes) spanning 82 Desikan-Killiany brain regions. We extracted 33 channel-level features including relative bandpower in canonical frequency bands (δ-γ), FOOOF toolbox (fitting oscillations & one over f) spectral parameterization including aperiodic slope and peak characteristics, spectral entropy, and 22 time-series metrics from the Catch22 package characterizing temporal dynamics including autocorrelation and distribution properties. We quantified batch effect magnitude via Cohen's d effect sizes and site classification AUC using logistic regression and random forest classifiers to assess separability between sites. We applied NeuroComBat harmonization to model site-specific batch effects while preserving biological covariates including brain region and spatial coordinates. We assessed clinical biomarker validity through resected versus spared tissue classification before and after harmonization within the Penn cohort. Inter-site pre-harmonization analysis showed substantial batch effects with perfect site classification (AUC = 1.000) and strong effect sizes (Cohen's d > 0.8 in 24/33 features).

ComBat achieved 90.8% median batch reduction with 28/33 features showing >80% reduction. Linear classifiers reached near-chance post-harmonization (logistic regression AUC=0.566; SVM=0.507) while nonlinear classifiers retained discriminability. Clinical biomarker validity was preserved with 30/33 features retaining ≥90% original effect sizes (low-frequency power: d=-0.627→-0.641; transition variance: d=-0.544→-0.552). Multi-feature classification improved (random forest AUC: 0.781→0.811), indicating successful noise reduction. FOOOF parameters showed strongest batch effects but highest harmonization gains (98.8% reduction); bandpower showed minimal effects. This protocol reduces multi-site iEEG batch effects while preserving clinical signals, supporting large-scale epilepsy studies and standardized normative EEG atlas development for epileptogenic network localization.

Disclosures: T. Okalova: None. **N. Sinha:** None.

Poster

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Topic: B.08. Epilepsy

Support: ANR-21-CE17-0029 DYNEUMICS

Title: Frequency-dependent neuronal modulation by electrical brain stimulation in human epilepsy: revealing remote effects at the single-unit scale

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Abstract: Electrical brain stimulations (EBS) play a crucial role in the pre-surgical evaluation of drug-resistant epileptic patients during stereo-electroencephalography (SEEG). EBS help assess tissue excitability and functional interactions to delineate epileptic networks and evaluate surgical options. However, their effectiveness is limited by false positives (unusual seizures) and false negatives (no effect in functional areas), which result from a still incomplete understanding of neuronal activation following EBS. This study aims to bridge the gap between current knowledge and clinical practice to better understand EBS mechanisms at a single-neuron scale, both on local circuits, and on remote excitation and inhibition cascades. We explored the impact of EBS on neuronal dynamics using hybrid electrodes with tetrodes that allow multiscale signal acquisition. We analyzed the variations of neuronal dynamics in terms of firing rates 10 s before and 10 s after EBS. Forty SEEG patients and over 4,770 EBS from the Toulouse Epilepsy Unit were included. We present here the preliminary analyses of three patients. A total of 187 EBS were applied in the frontal lobe, 14 in the temporal lobe, and 5 in the insula. Recordings were

obtained from 68 neurons in the frontal lobe, 47 in the temporal lobe, 27 in the insula, with respective median changes in firing rates of -8.11% (interquartile range (IQR): -57.9%, 52.8%), -12.0% (IQR: -52.63%, 40.0%), 0.0% (IQR: -47.6%, 63.7%). Raw firing rate changes show broad variability across neurons, with distributions spanning both strong excitatory and inhibitory responses. Statistical analysis by quantile regression reveals that the variation in response is significantly modulated by both EBS frequency and distance from the stimulation site. Specifically, remotely (in a different lobe), the variation is significantly lower at 50 Hz than at 1 Hz ($p = 0.003$), but this effect vanishes for local EBS. Local EBS at 1 Hz leads to a significantly lower variation than distant EBS ($p < 0.001$). In contrast, at 7 Hz, local variations are significantly higher than distant ones ($p = 0.031$). No statistical variations were found for EBS at 20 Hz. These findings support a frequency-dependent modulation of the excitation-inhibition balance by the topography of the stimulation. This is the first human study of the effects of EBS on neuronal dynamics recorded by tetrodes. It may help to better understand the impact of EBS in terms of excitation and inhibition and to refine clinical practice.

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Poster

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Program #/Poster #: PSTR061.12/C59

Topic: B.08. Epilepsy

Support: R01-NS094399
K01-ES026839
UL1-TR000433

Title: Seizure source localization in refractory epilepsy patients from ictal intracranial EEG data using dynamic mode decomposition

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Abstract: Epilepsy is a neurological disorder characterized by recurring unprovoked seizures. Medication alone does not control seizures in all patients. To attain seizure freedom, resective surgery remains the next best option for patients with focal refractory epilepsy. Yet, surgery does not always result in seizure freedom. Improvements in seizure source localization may have a positive impact on surgical outcomes. The purpose of this study was localization of seizure activity from iEEG data using dynamic mode decomposition (DMD) to compare with the clinically documented seizure onset zone (SOZ). Analysis was performed on retrospective data

from sEEG patients with poor surgical outcomes. Clinical seizures documented in the long-term monitoring reports were selected for analysis, and seizures with low data quality were redacted. Contacts known to be extraparenchymal or to have poor data quality were dropped. A common average reference was applied. A band pass filter was applied between 1 and 40 Hz. Independent Component Analysis was performed to reduce noise. The data was down sampled to 128 Hz to reduce computational cost. A thirty-second window of data was selected for analysis starting at clinically documented seizure onset. The data were then quantified using DMD to provide a low-dimensional representation of the high-dimensional complex seizure data (blind source separation). DMD was applied to 5 second epochs, with a 1 sample offset between epochs. The real part of the dominant mode was extracted for each epoch. The dominant mode was selected as the seizure was expected to be the dominant brain activity in the ictal window. A matrix was created for each seizure with a row per recording contact and a column per each epoch. Contacts in which outlier values occurred in the first two seconds following seizure onset were compared to the clinically documented SOZ. Using the binomial cumulative distribution, we assessed whether the percentage agreement was better than random chance, based on the total number of contacts and the number of SOZ contacts in each patient. Whether the percentage of seizures with statistically significant agreement was greater than random chance was computed for each patient, again using the binomial cumulative distribution. Statistically significant agreement ($p < 0.05$) was found in 5 sEEG patients (ongoing) with additional common contacts also identified across seizures. We concluded that DMD can identify the “seizure mode” of activity and may therefore aid in the analysis of seizure localization. Further analysis will show the extent to which DMD can assist in surgical planning and contribute to the optimization of resective surgery outcomes.

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Poster

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Program #/Poster #: PSTR061.13/C60

Topic: B.08. Epilepsy

Title: Fractional dynamics and closed-loop control for seizure prediction and intervention

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Abstract: Epilepsy is a major neurological disease affecting over 50 million people worldwide. Although there have been advancements in antiepileptic drugs and surgery, a significant number of patients remain unresponsive to these treatments. Accurate seizure detection opens a promising avenue for developing closed-loop neuromodulation therapies. In this study, we explored the use of fractional order dynamics for seizure prediction from intracranial EEG

(iEEG) recordings. We extracted three types of features: (1) eigenvalues and (2) alpha parameters from a fractional order system, and (3) Hurst exponents estimated via a long short-term memory (LSTM) network trained on synthetic fractional Brownian motion. For each 3-second sliding window, with a 1-second stride, we computed the mean and median of these features. The dataset consisted of iEEG records from 10 patients, totaling 35 ictal and 35 interictal blocks, with each recording sampled at either 500 or 512 Hz. Using a simple support vector machine (SVM) with a radial basis function kernel and 10-fold leave-one-patient-out cross-validation, we classified interictal versus ictal states. The Hurst exponent alone achieved the highest accuracy (~80%), while adding alpha and eigenvalue features did not improve performance. Interestingly, Hurst values were consistently lower during seizures, suggesting a loss of long-range temporal correlations in the ictal state. This finding is consistent with the hypothesis that seizure dynamics are characterized by increasing randomness and breakdown of structured neural activity. On the other hand, the alpha and eigenvalue parameters showed weaker separability between the ictal and interictal blocks and had higher variance within and across patients. Lastly, we implemented a control system that transitions ictal signals back toward interictal patterns upon seizure detection, simulating how neurostimulation could be applied in real time to suppress seizure activity. These findings highlight the value of Hurst exponents as robust features for seizure prediction and demonstrate the potential of fractional dynamics in EEG-based seizure modeling and control.

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Poster

PSTR061: Human Epilepsy

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Program #/Poster #: PSTR061.14/C61

Topic: B.08. Epilepsy

Title: Toward Personalized Neuromodulation for Epilepsy: Deep Learning Framework for Predicting Efficacy in Responsive Neurostimulation

Authors: E. GADO[1,2], P. HADAR[1], *S. S. CASH[3], P. SALAMI[1];
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Abstract: Drug-refractory epilepsy (DRE) affects approximately 35% of the 70 million people living with epilepsy worldwide. For patients ineligible for resective surgery, Responsive Neurostimulation (RNS) offers a promising alternative, though one in four patients do not see substantial benefit. Accurately predicting which patients will benefit from RNS could greatly improve patient selection and reduce trial-and-error in treatment planning. While recent studies have explored the potential of pre-implantation recordings for outcome prediction, traditional methods often fail to capture the complex network dynamics underlying RNS efficacy.To address this, we propose the first deep learning framework for predicting RNS outcomes using

intracranial seizure data. The response variable was the Engel outcome, assessed ≥1 year post-implantation and grouped into three classes: I-II (14 patients, good response), III (18, intermediate), and IV (17, minimal or no benefit). In order to extract predictive features from ictal brain networks, we combined intracranial EEG (iEEG) with clinical metadata detailing the brain regions where electrodes were implanted during monitoring and where RNS leads were later placed. We curated a dataset of 30-second iEEG segments centered on seizure onset from 387 seizures across 49 DRE patients who later received RNS. Functional Connectivity Networks (FCNs) were constructed using magnitude-squared coherence across broadband and frequency-specific (alpha, beta, gamma) bands.These networks informed preliminary graph-theoretical analyses and were combined with clinical metadata to generate graph-based representations for input to Graph Neural Network (GNN) architectures. Performance was evaluated under both patient-stratified and non-stratified cross-validation schemes. High accuracy in non-stratified settings (up to 94% balanced accuracy) underscores the expressiveness of FCN-derived graph representations and the suitability of GNNs for capturing seizure network dynamics. To enhance generalizability, we implemented a Domain Adversarial Learning (DAL) framework, which achieved up to 76.7% balanced accuracy and enabled patient-invariant classification, enhancing both robustness and interpretability.Although generalization to completely unseen patients remains an open goal, our findings offer a strong foundation for expanding this work. This framework suggests new methodological directions for integrating brain network analysis and machine learning,

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Poster

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Topic: B.08. Epilepsy

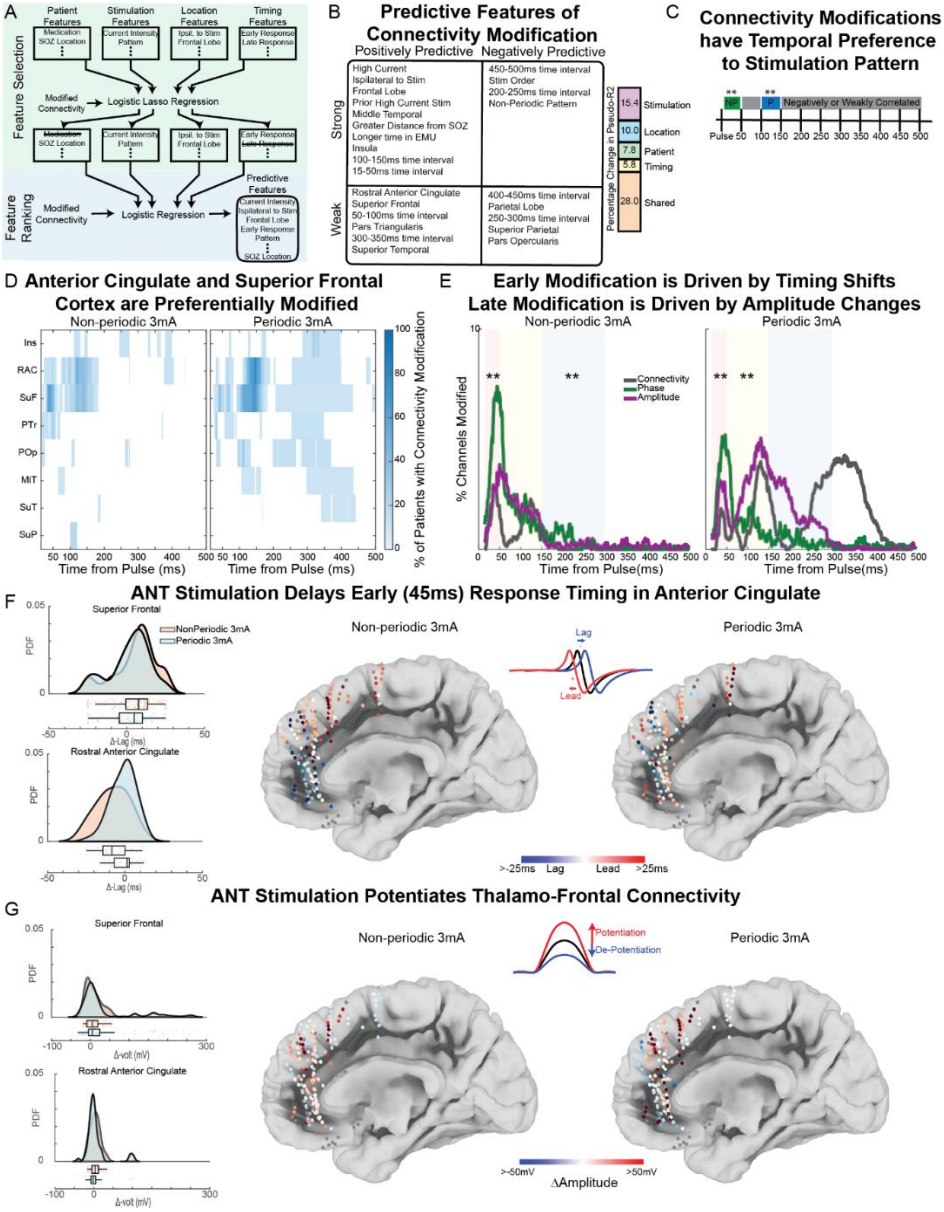
Support: Neurosurgery Research and Education Foundation

Title: Network Reorganization of Anteriorgrade Thalamic Connectivity During Novel Patterned DBS

Authors: *T. D. JOHNSON[1], J. J. PARKER[2], B. MOHAN[2];
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Abstract: Deep brain stimulation (DBS) of the anterior nucleus of the thalamus (ANT) can reduce seizures in drug-resistant epilepsy, but seizure freedom remains rare and no objective early-response biomarker exists. A short-term marker of therapeutic response could expedite DBS parameter optimization and improve seizure control. We investigated short-term ANT stimulation (minutes) in nine drug-resistant epilepsy patients undergoing intracranial EEG monitoring, comparing standard high-frequency periodic versus a novel non-periodic pulse

pattern. Stimulation intensities of 3mA altered the first 150ms of cortico-cortical evoked potentials (CCEPs) in thalamo-cortical circuits, with the anterior cingulate and superior frontal cortex showing the most consistent changes. Notably, non-periodic stimulation selectively modified early CCEP components relative to periodic stimulation with these changes being more related to changes in response phase. These findings highlight a pattern-specific modulation of connectivity between the ANT and spatially restricted cortical areas. When modeling what factors are most associated with CCEP changes, parameters related to the type of stimulation and location of stimulation were more predictive than patient-specific features. CCEP changes are quickly elicited, making them a potential treatment response biomarker for ANT-DBS epilepsy patients while in clinic. Prospective validation is required to determine if this biomarker can guide clinicians towards ideal parameters for ANT-DBS mediated seizure suppression.



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Poster

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Program #/Poster #: PSTR061.16/C63

Topic: B.08. Epilepsy

Title: Identification of Epilepsy Using Phase-Amplitude Coupling from Resting-State EEG Recorded with a Patch-Type Sensor

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Abstract: The diagnosis of epilepsy requires effort in many respects. Previous studies have explored various electrophysiological features of the electroencephalography (EEG) and magnetoencephalography (MEG) signals of patients with epilepsy. For instance, resting-state phase-amplitude coupling (PAC) has been shown to differ between patients with epilepsy in the interictal state and healthy individuals. In this study, we analyzed EEG signals measured using a patch-type EEG sensor to demonstrate that resting-state PAC differs between patients with epilepsy and healthy individuals. This study included 90 patients with various types of epilepsy and 46 healthy participants. Patients with epilepsy were subdivided into two groups: the severe epilepsy group includes 31 patients with more than 12 seizure days per year; the mild epilepsy group includes 59 patients with 12 or fewer seizure days per year. We used HARU-2, a patch-type EEG sensor (3 frontal polar channels, 1000 Hz sampling), to measure EEG data of the 10 minutes resting state. PAC was evaluated using the synchronization index (SI) for 10 frequency band pairs: the phases of δ (1-3 Hz), θ (4-7 Hz), α (8-13 Hz), low β (14-20 Hz) and high β (21-35 Hz), and the amplitudes of low γ (35-55 Hz) and high γ (65-90 Hz). Additionally, we used receiver operating characteristics (ROC) curve analysis to explore the discriminatory value of severe epilepsy patients and healthy participants. When phase-locked power at the δ-wave peak was averaged across each group, a peak increase was observed in healthy participants and the mild epilepsy group, whereas no such increase was detected in the left bipolar channel of the severe epilepsy group. When the low-γ amplitude was divided into six bins ($-\pi$ to π) of the δ-wave phase, significant differences among phases were observed in both channels for healthy participants and the mild epilepsy group ($p < 0.001$, one-way ANOVA), whereas no significant differences were found in either channel for the severe epilepsy group. Compared with healthy participants and the mild epilepsy group, the severe epilepsy group showed significantly smaller mean SI values for δ/low-γ band pairs in the left bipolar channel ($p < 0.05$, Wilcoxon rank sum tests, FDR). ROC curve analysis comparing healthy participants to the severe epilepsy group yielded an AUC of 0.74, while the comparison between the mild and severe epilepsy groups resulted in an AUC of 0.72. These results suggest that analyzing EEG signals measured using a

patch-type EEG sensor could potentially distinguish patients with severe epilepsy from healthy participants and mild epilepsy.

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Poster

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Title: Pre-ictal spectral dynamics may distinguish consciousness outcomes in childhood absence epilepsy

Authors: *A. A. SHARMA[1], A. KHALAF[1], S. SUMSKY[2], H. BLUMENFELD[2];
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Abstract: Spike-and-wave discharges in childhood absence epilepsy (CAE) can occur with or without consciousness impairment, but the neural mechanisms underlying this variability remain unclear. Understanding how pre-ictal brain state changes prepare for different seizure outcomes could provide insights into consciousness mechanisms. Pre-ictal arousal state is known to influence the likelihood of absence seizure occurrence, and recent work in animal models suggests that pre-ictal low-frequency cortical activity gradually increases for tens of seconds prior to seizure onset, whereas high-frequency activity increases in the same time period. Electroencephalogram (EEG) data were collected from 33 CAE patients (167 seizures, ~50% consciousness-spared) through two Yale CAE studies and the Danish Epilepsy Center. Consciousness status was determined by behavioral assessments during EEG monitoring. EEG data underwent preprocessing, including resampling (500 Hz), signal filtering (1 Hz high-pass, 60 Hz notch), re-referencing, and artifact removal. Pre-ictal spectral power was analyzed during the 30 s before seizure onset within five frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (30-60 Hz). Power was log-transformed and baseline-corrected using a 2-second reference period immediately preceding seizures. Analysis revealed a spectral crossover pattern where consciousness-impaired seizures showed higher delta but lower high-frequency power than consciousness-spared seizures in the 30 s before seizure onset. Specifically, delta and theta frequency power were greater for impaired seizures, whereas beta and gamma frequency power were greater for spared seizures. Alpha frequency power showed

no significant differences in the 30 s preceding seizure onset. These findings suggest that pre-ictal spectral patterns show frequency-dependent differences that distinguish consciousness outcomes in absence seizures. The spectral crossover from delta to gamma bands suggests different neural mechanisms prepare the brain for consciousness-sparing versus consciousness-impairing seizures. These pre-ictal spectral differences could reflect differences in arousal state, differences in seizure initiation mechanisms, or both, which influence subsequent electrophysiological and behavioral severity of seizures. With further investigation, understanding these mechanisms could lead to better treatments to predict and prevent consciousness-impairing seizures and improve quality of life.

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Poster

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Topic: B.08. Epilepsy

Support: NIH/NINDS UG3/UH3 NS112826

Title: Studying Involvement of Central Lateral Thalamus in Impaired Consciousness During Temporal Lobe Seizures

Authors: *T. YADAV[1], Z. ZHANG[2], V. KREMEN[5], K. L. CHRISTISON-LAGAY[8], E. C. DAMISAH[3], D. CORMIER[2], A. GUMMADAVELLI[9], V. SLADKY[6], J. HONG[10], K. BUJARSKI[11], G. MONCRIEF[12], J. L. BAKER[13], E. CHOI[14], J. M. HENDERSON[15], M. HOOK[16], B. BRINKMANN[6], N. GREGG[5], B. N. LUNDSTROM[6], I. H. QURAISHI[4], J. L. GERRARD[18], C. R. BUTSON[17], N. D. SCHIFF[19], B. C. JOBST[20], G. A. WORRELL[7], H. BLUMENFELD[8];
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Abstract: Impaired consciousness negatively impacts the quality of life for people with temporal lobe epilepsy (TLE). Prior animal and human studies have investigated the mechanisms of such impairment in the limbic and cortical structures but the involvement of intralaminar central lateral thalamus (CL) is unresolved. Current work focusses on quantitative analysis of

intracranial recordings of mesial temporal lobe seizures obtained simultaneously from hippocampal (HC) and CL thalamic leads, acquired by an investigational implanted neurostimulator with closed loop capabilities. Five patients with medically refractory mesial TLE were recruited and implanted for our ongoing clinical trial (Stimulation of the Thalamus for Arousal Restoral in TLE, START). All seizures had mesial temporal onset and received up to 5 sec of responsive HC stimulation (125 Hz, 90 or 120 μs) followed by 5 minutes of therapeutic CL stimulation (40 or 125 Hz, 90 or 120μs) at patient's tolerated current levels. During all seizures, patient's behavior was continuously assessed for ~7minutes using an Automatic Response Testing in Epilepsy (ARTiE) Watch and scored (0-3). Seizures were grouped into Focal Preserved Consciousness (FPC) and Focal Impaired Consciousness (FIC) based on their mean test score for the first three instructions after the seizure onset (mean score > 2.1 and <= 2.1 respectively). Intracranial EEG from HC and CL channels were preprocessed to identify and remove stimulation related artifacts. EEG power was computed in 1-sec bins using fast Fourier transform during baseline (30 sec), ictal (up to 370 sec) and postictal (60 sec) periods. Percentage change in power was computed relative to baseline. All seizures (n=45, 32 FIC, 13 FPC) showed dominant power in the beta band (14-25 Hz) for HC channels. Compared to FPC, FIC seizures displayed larger increases in HC power in beta, gamma (26-50 Hz) and alpha (8-13 Hz) bands. CL channels on the other hand had concurrent increases in delta (1-3 Hz) and gamma band power during the FIC seizures. Postictally, HC power returned to baseline level, but interestingly CL channels had sustained power in delta and gamma bands for FIC seizures. Quantitative analysis of TLE seizures so far points toward a complementary composition of HC and CL's EEG activity. Intralaminar thalamic slow wave activity may be representative of cortical slow wave activity observed previously in TLE. CL thalamic gamma activity during seizures is a novel finding and encourages further investigation to better understand mechanisms of impaired consciousness in focal epilepsy, and to improve treatment and quality of life.

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Poster

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Program #/Poster #: PSTR061.19/D2

Topic: B.08. Epilepsy

Support: K99NS133494

Title: Scalp EEG-based prediction of impaired consciousness in temporal lobe epilepsy using machine learning

Authors: *A. KHALAF[1], M. GRUEN[2], A. PRAT[3], A. YSMANOVA[4], E. MAKINDE[5], B. ABDALLA[2], N. SHAHANA[2], H. SUTHERLAND[2], G. A. WORRELL[6], H. BLUMENFELD[7]; [1]Yale Univ., New Haven, CT; [2]Yale Sch. of Med., New Haven, CT; [3]Yale Sch. of Med., New Haven, Chile; [4]Neurol., Yale university, New Haven, CT; [5]Ctr. for Neural Sci., New York Univ., Bowie, MD; [6]Mayo Clin., Rochester, MN; [7]Dept. of Neurol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Impaired consciousness in temporal lobe seizures poses significant challenges for patient safety and quality of life. Evaluating consciousness states during seizures remains difficult in clinical practice, especially when behavioral assessment is unavailable or seizures present with subtle behavioral changes. We implemented a machine learning approach to automatically detect consciousness impairment in temporal onset seizures through scalp EEG analysis, offering an objective diagnostic tool to support clinical decision-making. We developed a machine learning pipeline for analyzing scalp EEG data from temporal onset seizures (N=76 seizures, 35 with impairment). Our methodology utilized common spatial pattern (CSP) algorithm to extract spatial features that optimize discrimination between spared and impaired consciousness states. We also extracted time-domain and frequency-domain characteristics from pre-ictal and ictal periods. Classification was performed using support vector machines and linear discriminant analysis, with performance evaluated through 10-fold cross-validation. Information fusion from pre-ictal and ictal periods was achieved via feature vector concatenation and probabilistic fusion techniques. The system was designed to support clinical decision-making for medication adjustments, therefore, our objective was to maximize overall accuracy and obtain balanced performance across impaired and spared consciousness states. The machine learning classifier demonstrated 82.8% overall accuracy for consciousness state classification during temporal onset seizures, showing balanced sensitivity for spared (82.9%) and impaired (82.8%) consciousness states. Spatial features derived from CSP analysis were most effective for consciousness state discrimination, successfully capturing the complex spatiotemporal dynamics of temporal lobe seizure propagation. These findings establish that machine learning analysis of scalp EEG recordings can reliably predict consciousness impairment during temporal onset seizures. The balanced classification performance supports clinical applicability for informing treatment strategies, especially medication management decisions. Clinical deployment of this approach could offer physicians an objective consciousness assessment tool for temporal lobe epilepsy, with potential to enhance therapeutic precision and improve patient care outcomes.

Disclosures: A. Khalaf: None. **M. Gruen:** None. **A. Prat:** None. **A. Ysmanova:** None. **E. Makinde:** None. **B. Abdalla:** None. **N. Shahana:** None. **H. Sutherland:** None. **G.A. Worrell:** None. **H. Blumenfeld:** None.

Poster

PSTR061: Human Epilepsy

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR061.20/D3

Topic: B.08. Epilepsy

Support: NIH/NINDS UG3/UH3 NS112826

Title: Intralaminar Thalamic Stimulation Elicits Behavioral and Electrophysiological Arousal from Sleep: Parameter Titration for Epilepsy Therapy

Authors: *Z. ZHANG[1], T. YADAV[2], V. KREMEN[5], K. DACOSTA[1], D. CORMIER[1], K. L. CHRISTISON-LAGAY[8], E. C. DAMISAH[3], A. GUMMADAVELLI[9], D. D. SPENCER[10], V. SLADKY[6], J. HONG[11], K. BUJARSKI[12], G. MONCRIEF[13], R. H. ROTH[1], J. L. BAKER[15], E. CHOI[16], J. M. HENDERSON[17], M. HOOK[18], B. BRINKMANN[6], N. GREGG[5], B. N. LUNDSTROM[6], I. H. QURAISHI[4], J. P. ARONSON[19], J. L. GERRARD[20], J. VAN GOMPEL[7], C. R. BUTSON[21], N. D. SCHIFF[22], B. C. JOBST[14], G. A. WORRELL[6], H. BLUMENFELD[8]; [2]Neurol., [3]Neurosurg., [1]Yale Univ., New Haven, CT; [4]Dept. of Neurol., Yale Univ., NEW HAVEN, CT; [5]Neurol., [7]Neurosurg., [6]Mayo Clin., Rochester, MN; [8]Dept. of Neurol., [9]Yale Univ. Sch. of Med., New Haven, CT; [10]Dept Neurosurg., Yale Univ. Sch. Med., New Haven, CT; [11]Dartmouth-Hitchcock, Hanover, NH; [12]Dartmouth Hitchcock Med. Ctr., Lebanon, NH; [14]Neurol., [13]Dartmouth-Hitchcock Med. Ctr., Lebanon, NH; [15]BMRI, Cornell University: Weill Cornell Med. Col., New York, NY; [17]Dept Neurosurgy, R 227, [16]Stanford Univ., Stanford, CA; [18]Univ. of Florida, Gainesville, FL; [19]Beth Israel Deaconess Med. Ctr., Cambridge, MA; [20]Dept. of Neurosurg., Yale Sch. of Med., New Haven, CT; [21]Norman Fixel Inst. for Neurolog. Dis., Univ. of Utah, Gainesville, FL; [22]Weill Cornell Med. Col., New York City, NY

Abstract: Thalamic intralaminar stimulation is under investigation as a strategy to restore consciousness during temporal lobe seizures. To optimize stimulation parameters, we employed arousal from sleep as an indicator of arousal efficacy. This approach also offers a unique opportunity to examine the effects of bilateral central lateral (CL) thalamic stimulation on natural sleep-related arousal in humans. As part of the ongoing clinical trial, Stimulation of the Thalamus for Arousal Restoral in Temporal Lobe Epilepsy (START), five patients with medically refractory temporal lobe epilepsy were implanted with an investigational implantable neural sensing and stimulation device (INSS), targeting the CL nucleus of the thalamus and the bilateral hippocampi. During inpatient video-scalp EEG monitoring, an overnight sleep session was conducted with simultaneous intracranial recordings. The maximum tolerated CL stimulation current (the highest amplitude without adverse effects) was established during wakefulness. Sleep stages were continuously tracked via scalp EEG, and stimulation was remotely delivered during N2 or N3 non-rapid eye movement sleep. CL stimulation was administered in 5-minute epochs throughout the night, with systematic variation in current amplitude, frequency (40 or 125 Hz), and pulse width (90 or 120 μs). Stimulation parameters and electrode contacts were individualized using computational modeling. Arousal responses were quantified by changes in EEG spectral power, respiratory rate, and body movements relative to a 5-minute pre-stimulation baseline. Results showed that across patients, CL stimulation resulted in significant reductions in delta (1-3 Hz) and theta (4-7 Hz) band power, calculated from the most effective stimulation trial per patient, relative to baseline (mean reductions of 8 dB and 5.9 dB, respectively; $p < 0.05$, Wilcoxon rank-sum test). These spectral changes were accompanied by increased respiratory rate and body movements, indicative of transient arousal. In two patients, stimulation elicited a clear transition from N2 sleep to wakefulness on EEG, while the remaining three exhibited stage shifts from N3 to N2 or N2 to N1. All patients subsequently returned to sleep, as verified by both EEG and behavioral observations. These findings indicate that stimulation of the CL nucleus reliably induced transient arousal from sleep, marked by

reductions in delta and theta power alongside increased physiological and behavioral arousal indices. These results underscore the CL's role in arousal regulation and its potential as a neuromodulatory target for impaired consciousness in epilepsy and other disorders.

Disclosures: Z. Zhang: None. **T. Yadav:** None. **V. Kremen:** None. **K. DaCosta:** None. **D. Cormier:** None. **K.L. Christison-Lagay:** None. **E.C. Damisah:** None. **A. Gummadavelli:** None. **D.D. Spencer:** None. **V. Sladky:** None. **J. Hong:** None. **K. Bujarski:** None. **G. Moncrief:** None. **R.H. Roth:** None. **J.L. Baker:** None. **E. Choi:** None. **J.M. Henderson:** None. **M. Hook:** None. **B. Brinkmann:** None. **N. gregg:** None. **B.N. Lundstrom:** None. **I.H. Quraishi:** None. **J.P. Aronson:** None. **J.L. Gerrard:** None. **J. Van Gompel:** None. **C.R. Butson:** None. **N.D. Schiff:** None. **B.C. Jobst:** None. **G.A. Worrell:** None. **H. Blumenfeld:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.01/D4

Topic: C.01. Brain Wellness and Aging

Title: Modeling Brain Aging in Space: A Multi-Omics Study of Age-Dependent Responses in Human Organoids

Authors: *W. GOLDMAN[1], **A. MARTINS**[2];
[1]Akoya Biosci., Marlborough, MA; [2]UCSD, San Diego, CA

Abstract: Spaceflight accelerates biological aging through a combination of microgravity, radiation, and systemic stressors, yet the effects of this space environment on neurodevelopment remain largely unexplored. In this study, we launched human brain organoids at three developmental timepoints— 2, 3, and 5 months of age—to the International Space Station to investigate how space-induced aging influences neural trajectories across maturation stages. Upon return, the organoids underwent comprehensive multi-omics analysis, including bulk proteomics, single-cell transcriptomics, and single-cell proteomics through proteomics-based Astral-Orbitrap mass spectrometry. To spatially resolve protein expression with cell-type and regional context, we leveraged spatial proteomics using Akoya Biosciences' PhenoCycler®-Fusion platform in combination with the PhenoCode™ Human Neurobiology Panel. This integrative approach is expected to reveal age-dependent molecular signatures of accelerated aging, highlighting developmental windows of heightened susceptibility. We demonstrate that earlier-stage organoids exhibit disruptions in neurogenesis and cytoskeleton structure, while more mature organoids showed changes in oxidative stress responses, synaptic architecture and inflammatory pathways. By modeling aging in space across developmental stages, this work aims to generate the first high-resolution molecular atlas of the human brain's response to space environmental conditions. These insights will enhance our understanding of brain aging and inform countermeasures for neurobiological risks associated with long-duration spaceflight.

Disclosures: **W. Goldman:** A. Employment/Salary (full or part-time): Akoya Biosciences, UC San Diego. **A. Martins:** A. Employment/Salary (full or part-time): UCSD.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.03/D5

Topic: C.01. Brain Wellness and Aging

Support: The National Priority Research Center Program (NRF-2021R1A6A1A03038865)
Bioimaging Data Curation Center Program (NRF-2022M3H9A2096198)
the Basic Science Research Program Grant (RS-2025-00514203)
National Center for Standard Reference Data, Korean Agency for Technology and Standards, Ministry of Trade, Industry and Energy, Ministry of Health and Welfare (grant HI12C1847; Korea Healthcare Technology R&D Project)

Title: Distinct Spatiotemporal Patterns of White Matter Hyperintensity Progression

Authors: ***J. CHUNG**[1,2], E. HADDAD[3,4], N. JAHANSHAD[3,4], H. KIM[3], D.-E. KIM[1,2,5];
[1]Dongguk Univ., Goyang-si, Korea, Republic of; [2]Natl. Priority Res. Ctr. for Stroke, Goyang, Korea, Republic of; [3]Mark and Mary Stevens Neuroimaging and Informatics Inst., [4]Imaging Genet. Ctr., USC, Los Angeles, CA; [5]Dept. of Neurol., Dongguk Univ. Ilsan Hosp., Goyang, Korea, Republic of

Abstract: White matter hyperintensity (WMH), a key imaging marker for brain health and the most prevalent brain abnormality in the general population, has prognostic implications for stroke. In this paper, we show three distinct spatiotemporal patterns of WMH progression in ischemic stroke patients. We used a multicenter MRI database of 9,719 consecutive patients with acute ischemic stroke, plus the UK Biobank database (n = 36,210 controls). Subtype and Stage Inference (SuStaIn) modeling was performed to simultaneously subtype and stage WMH based on longitudinal inference from the Korean multicenter cross-sectional data. Then, we rigorously characterized demographic profiles, vascular risk factors, and stroke outcomes across three different WMH progression trajectories: fronto-parietal (FP), radial, and temporo-occipital (TO) progression. Subtypes remained consistent between baseline and follow-up (n= 264, median [interquartile range] years of follow-up: 4.5 [2.8–7.3]) in the majority (> 77%) of patients during WMH progression. FP subtype showed relatively delayed WMH onset and was enriched for women and hypertension, whereas TO subtype showed earlier WMH onset and was enriched for men, atrial fibrillation, and coronary artery disease. Hypertension and diabetes mellitus were major risk factors that could accelerate WMH progression in all subtypes. Acute symptomatic infarction was more likely to be due to small vessel occlusion in FP subtype and cardioembolism in TO subtype. Regarding post-stroke outcomes, early (< 3 weeks) neurological deterioration by

symptomatic hemorrhagic transformation and 3-month unfavorable functional outcome were more frequent in TO subtype, while 1-year ischemic stroke recurrence was more frequent in FP subtype. We also found that our WMH subtyping-staging model and the widely used Fazekas WMH scale were consistent while also offering complementary strengths in profiling stroke risk factors and outcomes. Using our model, high-risk controls in the UK Biobank were assigned to their most likely WMH subtype and stage, demonstrating spatiotemporal patterns of WMH progression that closely paralleled those observed in stroke patients. Of note, we observed distinct stage distributions between high-risk controls and stroke patients, along with a higher performance for distinguishing stroke patients from high-risk controls, compared to that of WMH volume. In conclusion, we identified distinct spatiotemporal trajectories of WMH progression. This WMH subtyping-staging model can capture demographic and vascular risk factors and may provide useful predictions for stroke outcomes across different WMH subtypes and stages.

Disclosures: J. Chung: None. **E. Haddad:** None. **N. Jahanshad:** None. **H. Kim:** None. **D. Kim:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.04/D6

Topic: C.01. Brain Wellness and Aging

Support: Restracomp Postdoctoral Research Fellowship

Title: Brain aging defines a structural signature of outcomes after vagus nerve stimulation in children

Authors: *T. LATYPOV[1], H. SURESH[2], K. MITHANI[3], F. VENETUCCI GOUVEIA[2], G. M. IBRAHIM[2];
[1]Hosp. for Sick Children, Toronto, ON, Canada; [2]The Hosp. for Sick Children, Toronto, ON, Canada; [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: Vagus nerve stimulation (VNS) is an essential neuromodulatory therapy for children with drug-resistant epilepsy (DRE), yet clinical predictors of response remain poorly defined. Current clinical and electrophysiological markers often fail to capture large-scale brain network integrity, limiting their prognostic value and utility. Brain age modeling—a machine learning approach estimating biological brain age from structural MRI—offers a promising single-metric index of brain health. While extensively applied in adults, its relevance to pediatric neurodevelopment and treatment response in epilepsy has not been established.
We developed a pediatric-sensitive brain age model using T1-weighted MRI data from 2,623 healthy individuals (1.9-90 years), enriched for pediatric representation, to learn nonlinear developmental trajectories. The model, based on XGBoost regression and FreeSurfer-derived

anatomical features (n=338), demonstrated high accuracy ($R^2 = 0.93$, MAE = 3.5 years) on test sets and strong generalizability on an pediatric external validation set (n=363; MAE = 2.16 years). We applied the model to a multisite cohort of 126 children with DRE from the CONNECTiVOS registry, all of whom underwent VNS with pre-implantation MRI and one-year clinical follow-up. Brain age gap (BrainAGEgap) was computed as the difference between predicted and chronological age. We analyzed relationship between BrainAGEgap and responce to VNS, quality of life (QoLCE), and seizure severity (SSQ). Feature contribution was evaluated via SHAP analysis and PCA.Children with DRE showed significantly elevated BrainAGEgap compared to healthy peers (mean gap = +7.7 years, $p < 0.0001$), indicating early-onset structural aging. Non-responders to VNS exhibited higher preoperative BrainAGEgap than responders ($p = 0.01$). Quality of life outcomes mirrored these findings: higher BrainAGEgap was associated with lower preoperative QoLCE scores ($r = -0.58$, $p = 0.016$) and minimal clinically important improvement after the surgery ($p = 0.02$). Feature importance analysis revealed dominant contributions from the core components of the vagus afferent network, including the cingulate cortex, thalamus, prefrontal cortex, along with nucleus accumbens, and lateral ventricular volumes.
Our findings reveal that early-onset structural brain aging, as captured by BrainAGEgap, stratifies children with DRE by VNS treatment outcome and pre-surgical quality of life. BrainAGEgap reflects cumulative deviations from normative neurodevelopment across networks critical to neuromodulation, offering a biologically grounded insights for diagnostic and therapeutic guidance.

Disclosures: T. Latypov: None. **H. Suresh:** None. **K. Mithani:** None. **F. Venetucci Gouveia:** None. **G.M. Ibrahim:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Canadian Institutes for Health Research Project Grant. F. Consulting Fees (e.g., advisory boards); LivaNova PLC, Synergia Medical Inc., Medtronic Inc..

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.05/D7

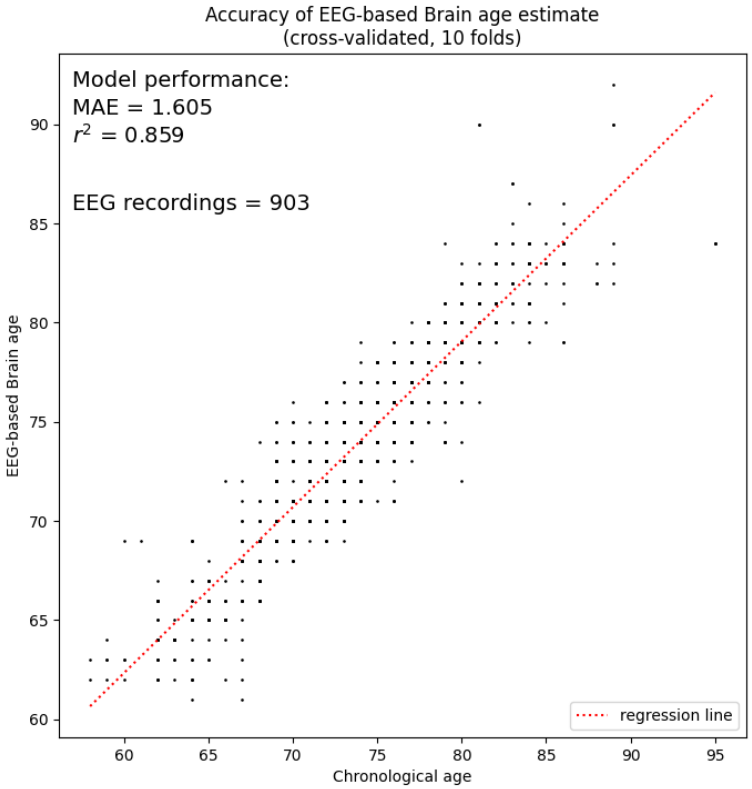
Topic: C.01. Brain Wellness and Aging

Support: GTM grant, Xjenza Malta

Title: Reading the brain’s age: High precision EEG-based neural age estimation for real-world applications

Authors: *J. DREO[1], B. MOHORCIC[2], T. PAVLOVCIC[2], A. DEMŠAR[2], A. OGRIN[2];
[1]Res. & Develop., [2]BrainTrip, Ljubljana, Slovenia

Abstract: Public and scientific interest in biological ageing has surged: the term's frequency in books has increased 4x over 50 years, while scientific publications have risen 10x, even after adjusting for overall literature growth. This indicates that research is not simply following a trend but actively shaping it. While MRI and PET have been used to estimate brain age, they remain costly and unsuitable for large-scale or longitudinal studies. EEG, by contrast, offers a scalable, low-cost alternative.We present the most accurate EEG-based brain age model to date, with MAE = 1.6 years and $R^2 = 0.86$, representing a 65% and 8% improvement, respectively, over the current state-of-the-art. The model was trained on 903 resting-state EEGs (24 channels) from 338 cognitively screened, neurologically healthy adults aged 58-95 (69% female) with diverse educational backgrounds. Recording quality was consistently maintained. The dataset, currently focused on older adults, will soon be expanded by ~1,000 younger individuals (ages 20-60), allowing full lifespan generalization. Full results will be available before September 2025. The final model draws on 133 confounder-corrected spectral features from 8 minutes of rest EEG (4 min eyes open, 4 min eyes closed). These features are fed into a machine learning algorithm to estimate brain age, which is then compared to chronological age for evaluation. The model is fully integrated into NeuroAI, a validated EEG platform that offers machine-assisted recording, automated artifact removal, feature extraction, confounder correction, and result computation, enabling point-of-care use. As opposed to previously described models, ours uniquely corrects for confounders like sex, education, health status, and head size. Unlike some reported model benchmarks, we emphasize raw model accuracy without regressing out chronological age. Our approach combines superior performance with rigorous validation and data quality controls, establishing EEG-based brain age as a reliable, practical biomarker ready for clinical, research, and lifestyle applications.



Disclosures: **J. Dreo:** A. Employment/Salary (full or part-time): BrainTrip. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BrainTrip. **B. Mohorcic:** A. Employment/Salary (full or part-time): BrainTrip. **T. Pavlovcic:** A. Employment/Salary (full or part-time): BrainTrip. **A. Demšar:** A. Employment/Salary (full or part-time): BrainTrip. **A. Ogrin:** A. Employment/Salary (full or part-time): BrainTrip.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.06/D8

Topic: C.01. Brain Wellness and Aging

Support: NIH R01NS121040

Title: Spatiotemporal characterization of negative hemodynamic response function across the human cortex with age

Authors: *N. J. FESHARAKI, A. VINOGRADOV, H. CHOI, J. KIM;
Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: In functional magnetic resonance imaging (fMRI), the blood oxygen-level dependent (BOLD) response to brief (<4s) neural activity is known as hemodynamic response function (HRF). Assuming linearity, positive HRF (pHRF)-an increased response following stimulus onset-is used for fMRI data analysis. The negative BOLD response is thus identified by a negative peak amplitude (PA), characterizing the negative HRF (nHRF) merely as an inverted pHRF. However, we previously showed that the nHRF is not only an inverted pHRF but has more variable dynamics. Here, we examined its dynamics across 10 young (22-35 yr) vs. 10 old (55-72 yr) cerebral cortices. We measured 80 (16 events x5 rep) HRFs using 3T fMRI with an event-related paradigm. Each event was a 2s audio-visual-motor task followed by a 28s fixation task. After preprocessing, each timeseries was gray matter depth averaged and mapped onto gray/white matter interface vertices. Using a model-free approach, HRFs were extracted and characterized by an initial negative response (INR)-first negative value with contrast-to-noise ratio (amplitude/its SEM) >1 and a PA within 2-14s. HRFs with a detectable INR identified as nHRFs. Smoothed HRFs were normalized and transferred into the standard MNI space. We temporally averaged HRFs across subjects for group analysis and used Dice coefficient for similarity assessment. nHRFs exhibited 3 distinct dynamics: 1) transient if PA >INR; 2) full-I (FI) if INR >PA; and 3) full-II (F-II) if only INR (Fig.1A). Old brains showed less nHRFs-particularly transient and F-I (Fig.1B). Spatial variations of both pHRF and nHRF were also smaller in old than young brains (Fig.1C). A relatively strong similarity (Dice coefficient =0.78) was obtained between their spatial distributions of HRF types (Fig.1D). nHRFs exhibit more complex and variable dynamics. We found spatial similarity coupled with a reduced nHRF proportion and a shift from transient and F-I nHRFs to F-II nHRFs or pHRFs with age, which may provide a valuable foundation for further investigation of data from patients with pathologies that affect HRF dynamics.

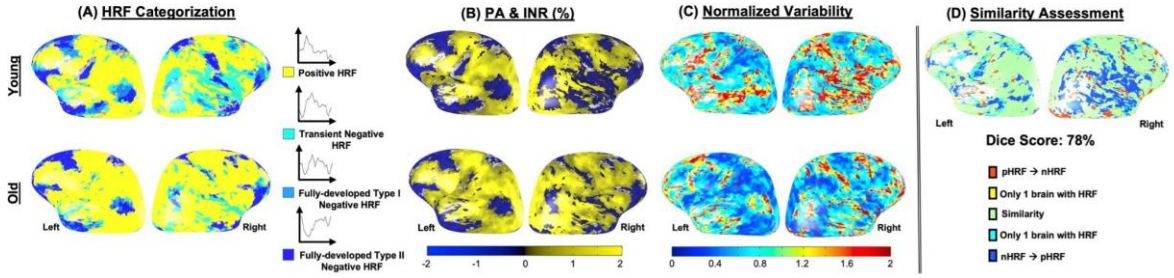


Figure. 1. HRF categorization (**A**), peak amplitude (PA) and initial negative response (INR) (**B**) and normalized variability (**C**) maps, and similarity assessment (**D**) between young and old brains.

Disclosures: N. J. Fesharaki: None. **A. Vinogradov:** None. **H. Choi:** None. **J. Kim:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.07/D9

Topic: C.01. Brain Wellness and Aging

Support: Canadian Institutes of Health Research (CIHR) operating grant MOP180400, PI - Dr. N. Malykhin
CIHR Mid Career Investigator Prize in Research in Aging (MOP179758) PI - Dr. N. Malykhin

Title: Effects of healthy cognitive aging on major hippocampal metabolites: a single voxel MR spectroscopy study at 3T.

Authors: *N. MALYKHIN[1], P. SERES[2], M. CAHILL[1], J. SERRANO[3];
[2]Radiology, [3]Psychiatry, [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Objective: The hippocampus has unique neuronal cytoarchitecture, capacity for neurogenesis, and a high degree of synaptic plasticity. Proton MR spectroscopy (1H-MRS) allows in vivo quantification of levels of brain metabolites, which serve as surrogate markers for different functions or cellular states. However, it remains to be determined whether healthy cognitive aging is associated with an impaired metabolic activity in the human hippocampus. Therefore, the main goal of the present study was to determine how age affects hippocampal metabolism across the adult lifespan.
Materials and methods: We recruited 117 (66 females, 51 males; 20-93 years old) healthy individuals. 1H-MRS was performed to measure metabolic concentrations for a single voxel VOI (40x13x17 mm^3, AP/IS/LR) in the right hippocampus on a 3T Siemens Prisma scanner. Unsuppressed water spectrum was acquired as well for quantification (H_2O). Spectral processing and analysis was performed with Osprey. The total of eight metabolites: N-acetyl-aspartate (NAA), total creatine (tCr), choline (Cho), myo-inositol (m-Ins), scyllo-inositol (s-Ins), glutamate (Glu), glutamine (Gln), and glutathione (GSH). Both metabolite ratios to tCr and H_2O were evaluated. Linear regression analysis and Pearson correlation were used to investigate the associations between age and metabolite ratios.
Results: Metabolite ratios of NAA/tCr (Pearson $r = -.194$, $p = 0.037$), Glu/tCr ($r = -.443$, $p < 0.001$), and GSH/tCr ($r = -.200$, $p = 0.035$) significantly decreased with age, while m-Ins/tCr ($r = .357$, $p < 0.001$) and s-Ins/tCr ($r = .404$, $p < 0.001$) ratios increased with age. Gln/tCr ratio was not affected by age. We found that tCr level showed a trend toward significant increase with age ($r = .181$, $p = 0.051$). Following the normalization of metabolites to H_2O, only m-Ins/H_2O ($r = .422$, $p < 0.001$) and s-Ins/H_2O ($r = .455$, $p < 0.001$) ratios demonstrated significant increase with age, while Cho/H_2O ratio showed a trend toward significant increase with age ($r = .166$, $p = 0.077$). Other metabolite ratios remained stable with age.
Conclusion: Age-related increases of tCr, m-Ins, and s-Ins were found in the hippocampus, indicating possible gliosis in the ageing brain. Since glial cells appear to act as a source of tCr but not NAA, an increase in glial cell number could explain the age-related tCr increase and NAA/tCr decrease. No age-dependent decreases in NAA/H_2O, Glu/H_2O, Gln/H_2O ratios were observed in the hippocampus indicating that no measurable loss in the amount of hippocampal neurons occurs in healthy aging. Our findings also imply that caution should be applied when using tCr as an internal reference to express metabolite changes in 1H-MRS.

Disclosures: N. Malykhin: None. **P. Seres:** None. **M. Cahill:** None. **J. Serrano:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.08/D10

Topic: C.01. Brain Wellness and Aging

Support: Canadian Institutes of Health Research (CIHR) Project Grant operating grant MOP180400
CIHR Mid Career Investigator Prize in Research in Aging (MOP179758)

Title: Contributions of hippocampal white matter connections to episodic memory function in healthy cognitive aging: a 4.7T Diffusion Tensor Imaging tractography study.

Authors: *G. NASR[1], J. SERRANO[2], N. MALYKHIN[2];
[1]Neurosci. and Mental Hlth. Inst., [2]Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Background: The fimbria, fornix, and parahippocampal cingulum (PHC) are the major hippocampal white matter tracts. Healthy aging affects hippocampal structure, its connectivity, as well as episodic memory performance. Previous diffusion tensor imaging (DTI) studies analyzed the fornix and fimbria as a single tract, masking potential contributions of each white matter tract to memory function. The main goal of this exploratory study was to investigate how age-related changes in white matter integrity of the fornix, fimbria, and PHC correspond to volumetric reduction in hippocampal subfields through the adult lifespan. The second goal was to examine how hippocampal white matter tracts support episodic memory function across the adult lifespan.
Methods: We recruited 125 healthy volunteers (18-85 years old). T2-weighted and DTI datasets were acquired on a 4.7T scanner. We performed deterministic tractography using DSI Studio to estimate mean length (ML), volume, fractional anisotropy (FA), axial (AD) and radial (RD) diffusivities. Hippocampal subfields (subiculum (Sub), cornu ammonis (CA1-3), and dentate gyrus (DG)) were manually traced using reliable volumetric protocol. Memory performance was assessed using the Weschler Memory Scale, 4th edition. Regression modeling and Pearson correlations were used to examine the links between age, DTI metrics, subfield volumes, and memory scores.
Results: ML and FA in the body and crus of fornix declined linearly with age (ps<0.05). Volume of the body of fornix rose until 30 years old and then declined (r=0.477, p<0.001), while the volume of crus declined linearly with age (r=0.219, p=0.008). Fimbria volume, ML, and FA started to decline after 50 years of age (ps<0.001). AD and RD increased simultaneously in the body and crus of fornix and fimbria following a quadratic fit (ps<0.05). ML and volume of the PHC were unaffected by age (ps>0.05), but FA declined linearly (r=0.197, p<0.026). AD and RD of the PHC increased after 60 years of age (ps<0.05). Sub and CA1-3 volumes were positively correlated with the volumes of fimbria, crus of fornix, and PHC (ps<0.05). DG volume was related to the volume of the body of fornix (r=0.229, p=0.011). When co-varying for age effects, visuospatial memory performance was linked to body of fornix ML (r=0.2, p=0.027).

In addition, body of fornix AD (r=0.349) and PHC FA (r=0.317) predicted verbal memory performance in males (*p*s<0.05).
Conclusions: Our results outline the differential impact of healthy aging on hippocampal white matter tracts. Our findings provide initial evidence of associations between the hippocampal subfields, their white matter tracts, and episodic memory function.

Disclosures: G. Nasr: None. **J. Serrano:** None. **N. Malykhin:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.09/D11

Topic: C.01. Brain Wellness and Aging

Support: National Institute on Aging (R01 AG054076, R01 AG049607, U01 AG052409, R01 AG059421, RF1 AG063507, RF1 AG066524, U01 AG058589)
National Institute of Neurological Disorders and Stroke (R01 NS017950 and UH2 NS100605)

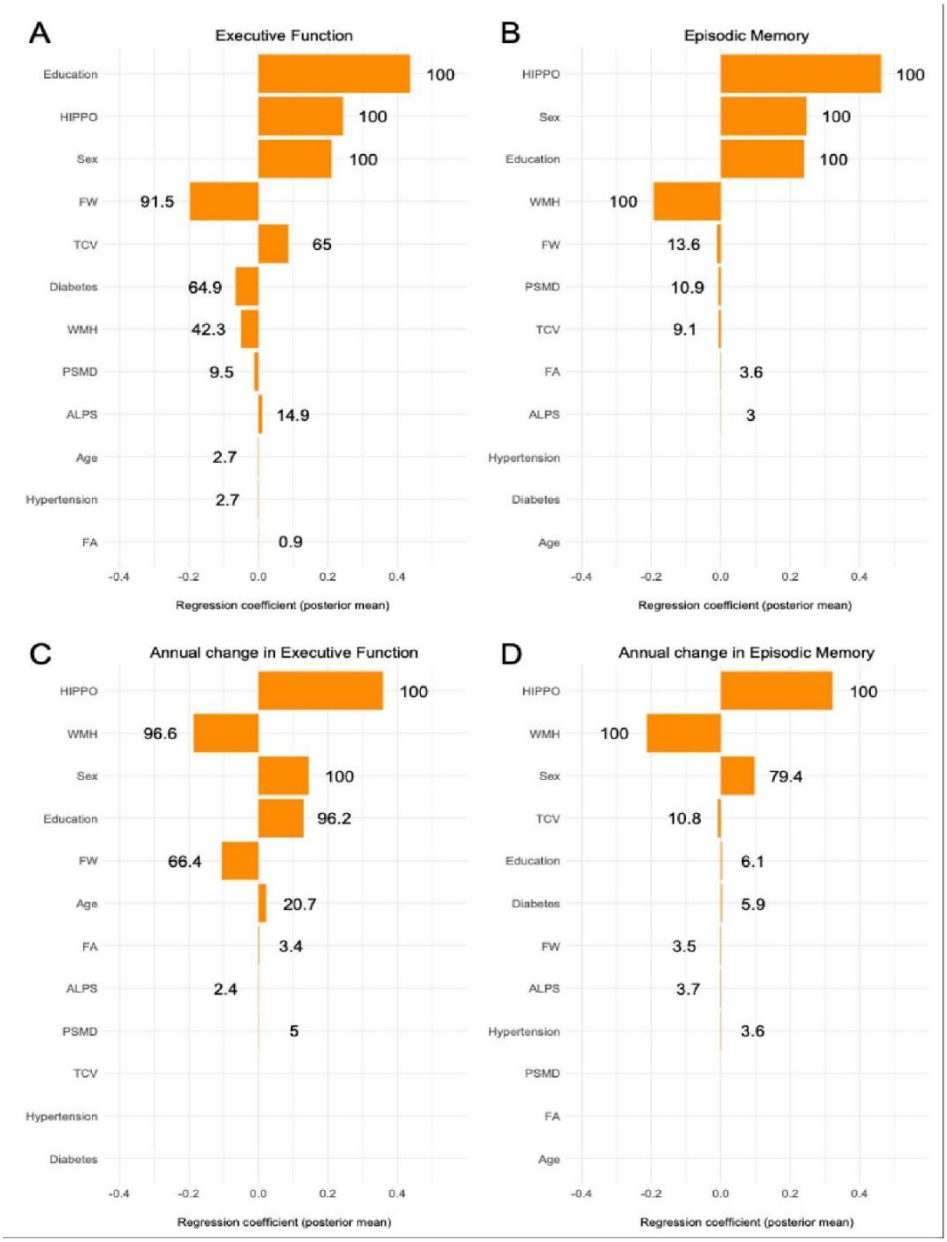
Title: Disentangling the Contributions of Cerebrovascular-Related White Matter Integrity Markers to Cognitive Aging

Authors: *E. AGAH[1], C. DECARLI[1], P. MAILLARD[2];
[1]Univ. of California, Davis, Davis, CA; [2]Neurol., Univ. of California Davis, Davis, CA

Abstract: Background: White matter (WM) integrity is essential for cognitive functioning, but the relative and joint contributions of various neuroimaging markers remain unclear. We evaluated five WM integrity markers, white matter hyperintensities (WMH), extracellular free water (FW), fractional anisotropy (FA), peak width of skeletonized mean diffusivity (PSMD), and diffusion tensor image analysis along the perivascular space (ALPS), in relation to cognitive performance and its trajectory in a cognitively diverse older cohort. **Methods:** 574 participants (77±7 years) from the UC Davis Alzheimer's Disease Research Center longitudinal cohort underwent baseline MRI. WMH, FW, FA, PSMD, and ALPS were used to model baseline and longitudinal change in episodic memory (EM) and executive function (EF) via linear regression with stepwise adjustment. Bayesian Model Averaging (BMA) was applied to quantify each marker's contribution while accounting for joint effects. **Results:** Higher WMH, FW, and PSMD, and lower FA and ALPS were associated with worse cognitive performance ($p<0.05$). These remained significant after adjusting for age, sex, education, hypertension, diabetes, and hippocampal volume, except FA and ALPS for EM ($p>0.05$). Higher WMH, FW, and PSMD, and lower FA, were linked to greater decline in EF and EM. ALPS was not associated with change ($p>0.05$). After adjustment, PSMD and FA were no longer linked to EM decline. BMA identified FW and WMH as the most probable contributors to both baseline performance and

cognitive change. **Conclusions:** This study provides a comprehensive comparison of five markers of WM integrity in relation to cognitive performance and decline in a cognitively diverse cohort of older adults. We found that FW and WMH emerged as the most probable candidates to explain the course of cognitive decline in EM and EF. The findings from the present study support the potential of combining multiple imaging metrics, particularly FW and WMH, to improve the early diagnosis and monitoring of cognitive decline.

Figure: Bayesian Model Averaging posterior mean and posterior inclusion probability



Summary of Bayesian Model Averaging models on baseline executive function (A) and episodic memory (B) and change in executive function (C) and episodic memory (D). Number next to orange bar indicates the marginal inclusion probability of the variable (see Methods). FW: free water content; FA: fractional anisotropy; PSMD: peak width of skeletonized mean diffusivity; ALPS: diffusion tensor image analysis along the perivascular space index; TCV: total cranial volume.

Disclosures: E. Agah: None. **C. DeCarli:** None. **P. Maillard:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.10/D12

Topic: C.01. Brain Wellness and Aging

Title: Metabolic regulation of neurovascular dysfunction and motor impairment in aging mice

Authors: *L. JIN[1], Z. CAI[1], Z. ZHAO[2];
[2]Physiol. and Neurosci., [1]USC, Los Angeles, CA

Abstract: Metabolism plays a vital role in regulating gene expression and protein function through post-translational modifications (PTMs), such as phosphorylation, acetylation, and methylation, particularly in neurodegenerative diseases. In this study, we observed significant age-related molecular changes in the perivascular regions of aging mice and these metabolic changes were accompanied by shifts in perivascular molecular profiles. Furthermore, treatment with 2-deoxy-D-glucose (2-DG), a glycolysis inhibitor, improved motor function in aging mice. These findings suggest a link between metabolic dysregulation, neurovascular dysfunction, and motor decline in aging, highlighting the therapeutic potential of targeting metabolic pathways in age-related neurological disorders.

Disclosures: L. Jin: None. **Z. Cai:** None. **Z. Zhao:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.11/D13

Topic: C.01. Brain Wellness and Aging

Support: Intramural Research Programs of the National Institute on Drug Abuse

Title: Sigma-1 receptor promotes glycolysis in neuronal systems by suppressing GRIM19 and modulating glucose transport

Authors: *S. COULY[1], Y. YASUI[1], Y. KIMURA[2], H.-E. WU[2], J. L. GOMEZ[2], M. MICHAELIDES[2], B. K. HARVEY[2], T.-P. SU[2];
[1]NIH/NIDA, Baltimore, MD; [2]NIDA/NIH, Baltimore, MD

Abstract: Sigma-1 receptor (S1R) is a Ca^{2+} sensitive, ligand-operated receptor chaperone protein present on the endoplasmic reticulum (ER) membrane and more specifically at the mitochondria-associated ER membrane (MAM). Upon activation by ER calcium depletion or ligand binding, S1R can increase calcium efflux from the ER into the mitochondria by chaperoning IP3 receptor type3 (Ip3R3). Mitochondrial metabolism has an intricate relationship with glycolysis. Despite S1R affecting mitochondria, the relevance of S1R to glycolysis and its impact on the overall cellular energy metabolism is not known. This study utilizes wild-type (Wt) and S1R knockout (S1R KO) Neuro2a (N2a) cells and Wt and S1R KO mice for primary culture of cortical neurons studies and longitudinal *in-vivo* imaging. In this manuscript we describe the fundamental functions of S1R on glycolysis, mitochondrial activity and NAD^+/NADH metabolism, keystone coenzymes essential for glycolysis and for mitochondrial activity. Both N2a cells and cortical neurons lacking S1R had reduced glycolytic activity, and increased mitochondria complex I protein GRIM19 but no change in mitochondrial oxygen consumption. Furthermore, we observed an increased NAD^+/NADH ratio in S1R KO condition. Positron emission tomography revealed decreased [^{18}F]fluorodeoxyglucose brain uptake in S1R KO mice. We observed that knocking down GRIM19 in S1R KO condition rescued the glycolysis deficit. Altogether, these data show for the first time that S1R modulates glycolysis and NAD metabolism in various neuronal systems. This new insight on the S1R function may lead to new therapeutic applications of S1R ligands where compromised glycolysis and cellular NAD^+/NADH ratios occur such as aging and neurodegeneration.

Disclosures: S. Couly: None. **Y. Yasui:** None. **Y. Kimura:** None. **H. Wu:** None. **J.L. Gomez:** None. **M. Michaelides:** None. **B.K. Harvey:** None. **T. Su:** None.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.12/D14

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant NS122808

Title: Recurrent hypoglycemia exposure impairs cognition in insulin-treated diabetic rats

Authors: *A. K. REHNI[1], S. MALLEPALLI[1], E. PEREZ LAO[1], F. FERRIER[1], M. A. PEREZ-PINZON[2], K. R. DAVE[1];
[1]Neurol., Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Univ. of Miami Sch. of Med., Miami, FL

Abstract: Approximately 38 million people in the USA have diabetes, and long-standing diabetes predisposes patients to worse cognitive outcomes. Antihyperglycemic therapy in diabetes causes transient hypoglycemia, which enhances the risk of recurrent hypoglycemia (RH). A study has shown that RH worsens cognitive function in diabetic rats[1]. Additionally, our

laboratory has shown that RH exposure to insulin-treated diabetic (ITD) rats impairs spatial learning and memory, as assessed using the Barnes circular platform maze test[2]. In the present study, we determined the effect of chronic (twice weekly for 4 weeks) RH exposure on non-spatial declarative recognition memory, spatial short-term working memory, and associative learning in ITD rats. We hypothesize that RH exposure leads to cognitive impairment in treated diabetes. Young male streptozotocin-diabetic rats received subcutaneous insulin pellet treatment 2-3 weeks after the onset of diabetes. The groups were randomly assigned to either hyperinsulinemic euglycemia (control) or hyperinsulinemic hypoglycemia (RH) groups. RH exposure was started after 2-3 weeks of insulin treatment. A supplemental subcutaneous injection of insulin was used to induce non-severe hypoglycemia twice weekly for 3 hours for 4 weeks. Behavioral assessment was started one week after the last episode of hypoglycemia. The effect of RH exposure on cognitive functions in ITD rats was assessed in terms of general activity, recognition memory, spatial short-term working memory, and associative learning, using an open field test (day 1), novel object recognition test (day 2-3), Y-maze spontaneous alternation test (day 4), contextual fear conditioning test (day 5-6), and cued fear conditioning test (day 7), respectively. The novel object recognition test results showed that the RH-exposed ITD rats (0.69±0.04, n=10) had a significantly decreased ($p<0.05$) discrimination index when compared to their hyperinsulinemic euglycemia control rats (0.82±0.02, n=8). Our results show that 4-week RH exposure impairs recognition memory in ITD rats when determined within the second week after the last episode of hypoglycemia. The results of the open field test, Y-maze test, and fear conditioning test are being analyzed. In the future, we intend to study the mechanism by which RH exposure induces cognitive impairment in ITD rats. References: 1) J. Neuroinflammation. 2012; 9: 182. 2) Transl. Stroke Res. 2019; 10(1): 78-90. Acknowledgement: NIH (NS122808).

Disclosures: **A.K. Rehni:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine. **S. Mallepalli:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine. **E. Perez Lao:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine. **F. Ferrier:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine. **M.A. Perez-Pinzon:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine. **K.R. Dave:** A. Employment/Salary (full or part-time): University of Miami Miller School of Medicine.

Poster

PSTR062: Brain Aging Analyses and Metabolism

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR062.13/D15

Topic: C.01. Brain Wellness and Aging

Support: Department of Biotechnology (DBT), Govt of India
Ramalingaswami Research Grant

Title: Inositol hexakisphosphate kinase-2 regulates energy dynamics and mitophagy via creatine kinase-B and PINK-1

Authors: *L. NAGPAL;
Univ. of Calcutta, Kolkata, India

Abstract: Inositol-hexakisphosphate kinases (IP6Ks) regulate various biological processes. Among pyrophosphates generated by IP6Ks, diphosphoinositol pentakisphosphate (IP7), and bisdiphosphoinositol tetrakisphosphate (IP8) have been extensively characterized. IP7 is produced in mammals by a family of inositol-hexakisphosphate kinases, IP6K1, IP6K2, and IP6K3, which have distinct biological functions. Among these, IP6K2 is highly expressed in the brain cerebellum. However, the function of IP6K2 is not well studied. To explore roles for IP6K2 in brain functions, we elucidated its protein interactome revealing a robust association of IP6K2 with creatine kinase-B (CK-B), a key enzyme in energy homeostasis. Cerebella of IP6K2-knockout [KO] mice produced less phosphocreatine and ATP and generated higher levels of reactive oxygen species and protein oxidative damage. In IP6K2-KO mice, mitochondrial dysfunction was associated with impaired expression of the cytochrome-c1 subunit of complex III of the electron transport chain. We were able to reverse some of these effects by combined treatment with N-acetylcysteine and phosphocreatine. We demonstrated that IP6K2 is involved in attenuation of PINK1-mediated mitochondrial autophagy (mitophagy) in the brain. Up-regulation of dynamin-related protein (Drp-1), as well as increased expression of mitochondrial biogenesis markers (PGC1-α and NRF-1) in IP6K2-KO mice, point to the involvement of IP6K2 in the regulation of mitochondrial fission. Overexpressing IP6K2 as well as IP6K2-kinase-dead mutant in IP6K2-knockdown cells reverses the expression of mitophagy markers, demonstrating that IP6K2- induced mitoprotection is catalytically/kinase independent. Overall, our study reveals a key neuroprotective role of IP6K2 in preventing PINK1-mediated mitophagy in the brain as well as regulating energy homeostasis through its interaction with CK-B.

Disclosures: L. Nagpal: None.

Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.01/D16

Topic: C.01. Brain Wellness and Aging

Title: Converging behavioral effects of diverse psychedelics via shared serotonergic pathways: 5-MeO-DMT exhibits neuroplastic and therapeutic potential

Authors: *C. DUCHEMIN, **S. WAGNER**, L. PETER, E. POIRAUD, B. HUYARD, E. ANDRIAMBELOSON;
NEUROFIT, ILLKIRCH, France

Abstract: Psychedelics acting through 5-HT2A receptor agonism are emerging as promising psychoplastogens to counter cognitive deficits and stereotypic behaviors in Alzheimer's disease (AD). However, differences in receptor selectivity and pharmacokinetics may influence their therapeutic relevance. Notably, 5-MeO-DMT exhibits high affinity at 5-HT1A receptors, potentially contributing to its less visually hallucinogenic profile. In addition, recent reports indicate 5-MeO-DMT also acts as an agonist at melatonin receptors MT1 and MT2, which are implicated in circadian regulation, neuroprotection, and mood-related behaviors. We assessed the neuroplastic and behavioral effects of 5-MeO-DMT in direct comparison to the previously evaluated DOI and Psilocybin. Neuronal morphology was quantified in primary cortical neurons following treatment. In parallel, behavioral effects were evaluated in mouse models using the marble burying task (to assess repetitive behavior) and spontaneous alternation following scopolamine administration (to assess cognitive performance through cholinergic deficit model). 5-MeO-DMT significantly enhanced neurite number, total length, and branching, replicating the neuroplastic profile of DOI. It also reduced stereotypic marble-burying behavior and reversed scopolamine-induced cognitive deficits, closely matching the behavioral efficacy of DOI and Psilocybin. These shared behavioral effects are likely mediated by overlapping serotonergic mechanisms, particularly high-affinity agonism at 5-HT2A and 5-HT1A receptors. While 5-MeO-DMT uniquely engages MT1 and MT2 receptors, the role of melatonergic activity in these outcomes remains speculative and requires further investigation. These findings position 5-MeO-DMT as a robust psychoplastogen with convergent behavioral and neuroplastic efficacy, and a distinct receptor profile. Its fast pharmacokinetics, short acute psychoactive effect, strong 5-HT1A activity, and additional melatonergic properties may support its relevance as a clinically adaptable psychedelic. Importantly, 5-MeO-DMT achieved comparable functional outcomes despite brief exposure, suggesting that rapid induction of neuroplasticity is possible without prolonged exposure — potentially offering therapeutic benefits with reduced psychedelic burden. Further work is needed to delineate the contribution of melatonin receptor activation to its functional outcomes.

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Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.02/D17

Topic: C.01. Brain Wellness and Aging

Support: NIH NINDS K23-NS124688
Tianqiao and Chrissy Chen Institute

Title: Gait Age Gap: A Quantitative Biomarker To Assess Cognitive Impairment From Gait

Authors: *S. YOU[1], L. BARNARD[1], J. GRAFF-RADFORD[1], D. S. KNOPMAN[1], C. R. JACK, JR.[2], P. VEMURI[2], R. PETERSEN[1], F. ALI[1];
[1]Neurol., [2]Radiology, Mayo Clin., Rochester, MN

Abstract: Gait age gap is a data-driven biomarker that estimates physiological ages based on gait characteristics, rather than chronological age. This approach quantifies subtle deviations in walking patterns, which may precede the onset of clinical cognitive impairment. We aim to develop a gait age prediction model and a gait-age gap index to measure gait alterations in older adults quantitatively. We utilized population-based individuals recruited by the Mayo Clinic Study of Aging, which included 20,001 pressure-sensitive walkway trials from 5,739 unique individuals. The age range of included individuals was 40-100 years old. The examined individuals did not have any medically diagnosed neurological or orthopedic cause for mobility impairment. 661 gaits parameters from a 10-meter walkway computed via the ProtoKinetics Movement Analysis Software were used as input variables. We regressed out the effects from covariates, such as sex, age, height, weight, and body mass index. We trained the least absolute shrinkage and selection operator regression to predict age from the gait parameters and measured the difference from chronological age as the gait age gap index. We used 1,502 cognitively unimpaired (CU) subjects with single-visit to normalize gait parameters via Z-transform and optimized model hyperparameters, while multiple-visit subjects were used for model evaluation and outcome analysis. Among 3,251 multiple-visits CU subjects, we sampled age-matched 569 subjects for group analysis and used the remaining 2,682 subjects to train and evaluate the gait age prediction model via leave-one-out cross-validation. We performed a cross-sectional group analysis to confirm the relevance of the gait age gap with cognitive impairment by comparing the gait-age gap index from 569 CU individuals, 516 with mild cognitive impairment(MCI), and 53 with dementia. We performed longitudinal Cox proportional hazard modeling to quantify the risk of conversion to cognitive impairment. Our gait age prediction model achieved an R^2 of 0.40 and MAE of 6.4 years, outstanding the velocity-only model with an R^2 of 0.25 and MAE of 7.3 years with 2,682 CU individuals. Increases in the gait age gap were observed in individuals with MCI (3.6±5.7 years) and dementia (5.7±5.7 years) compared to CU individuals (0.0±5.2 years). The gait age gap at baseline was significantly associated with increased risks of conversion to a cognitively impaired state with a hazard ratio of approximately 1.07, equating to a 7% increased risk per year of the gait age gap at baseline ($p < 0.005$). The gait age gap index is a promising tool to quantify effect of aging on gait and predict risk of future cognitive impairement.

Disclosures: S. You: None. **L. Barnard:** None. **J. Graff-Radford:** 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.; Biogen, Eisai. **D.S. Knopman:** 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.; Biogen, Lilly Pharmaceuticals. **C.R. Jack, Jr.:** None. **P. Vemuri:** None. **R. Petersen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oxford University Press, UpToDate. F. Consulting Fees (e.g., advisory boards); Roche, Inc., Genentech, Inc., Genentech, Inc., Nestle, Inc., Eisai, Inc.. **F. Ali:** None.

Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.03/D18

Topic: C.01. Brain Wellness and Aging

Support: NIA Grant R21AG08423

Title: Dual task gait performance in older adults without and with mild cognitive impairment

Authors: *V. PARIKH[1], J. NOCERA[2], M. E. HACKNEY[3], T. M. KESAR[2];
[1]Physical Therapy, Emory Univ. Sch. of Med., Alpharetta, GA; [2]Physical Therapy, Emory Univ., Atlanta, GA; [3]Emory University/Atlanta VAMC, Decatur, GA

Abstract: Background: Community ambulation relies on integrated motor control, sensory feedback, balance, and adaptability. These processes deteriorate with aging and cognitive decline as in mild cognitive impairments (MCI) and Alzheimer's disease (AD), reducing mobility and participation. **Objectives:** (1) To characterize differences in spatial and temporal gait variability as well as cost of cognitive dual task during gait among HOA, MCI, and AD groups; (2) To determine if locomotor and dual task deficits are correlated with cognitive and functional impairments. **Methods:** To date, 21 participants (HOA and MCI) were recruited in this study (22% male; age=70.6±5.3years). All participants underwent a baseline battery of clinical motor and cognitive assessments prior to performing a split-belt treadmill locomotor adaptation task. Dual task cognitive assessments (Serial 7's subtraction, phonemic fluency) were performed during 30 second treadmill walking bouts at self-selected gait speed, and gait kinetics and kinematics were assessed using motion capture. Dual task gait performance was compared between the HOA and MCI groups using independent samples t-tests. Pearson's correlation analysis was used to determine association between dual task and clinical gait performance. **Results:** Preliminary results from 6 participants (3 HOA, 3 MCI) showed that during dual task performance, people with MCI showed a greater dual task cost as compared to the HOA group. The MCI group showed a greater stride-to-stride variability in temporal gait parameters such as double support duration, stride, and step duration ($p > 0.05$). No significant differences were noted in the stride-to-stride coefficient of variance for spatial gait parameters such as step asymmetry between HOA and MCI. Individuals with MCI demonstrated significantly greater errors in the serial 7 subtraction dual task test ($p = 0.02$). Dual task performance on the Cognitive Timed Up and Go ($p=0.01$) and serial 7 subtraction during treadmill walking ($p=0.02$) were significantly correlated to motor performance on 10 meter walk test. Ongoing analyses are evaluating step length and kinematic variability during dual task walking as well as split belt adaptation, and processing the larger sample data (15 HOA, 15 MCI, 5 AD). **Conclusion:** Our results suggest that the cost of cognitive dual task during gait is greater in the MCI group as compared to the HOA group. The locomotion deficits and dual task deficits are associated with clinical functional mobility outcomes. Findings from this study will help elucidate the determinants of cognitive-motor deficits in older adults, regardless of cognitive status.

Disclosures: V. Parikh: None. **J. Nocera:** None. **M.E. Hackney:** None. **T.M. Kesar:** None.

Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.04/Web Only

Topic: C.01. Brain Wellness and Aging

Title: Hippocampal volume is associated with cognitive performance in patients with Parkinson's disease.

Authors: *J. ESQUIVIAS[1], C. PONCE FERNÁNDEZ[2], S. ALCAUTER[3];
[1]Univ. Nacional Autónoma de México, Querétaro, Mexico; [2]Univ. Anahuac Queretaro, Queretaro, Mexico; [3]Neurobiología Conductual y Cognitiva, Inst. De Neurobiologia. Univ. Nacional A, Queretaro, Mexico

Abstract: Cognitive impairment is a common condition in Parkinson's disease that leads to significant impairments in quality of life. The hippocampus is crucial for learning, memory and spatial navigation, among other relevant cognitive abilities. In this work, this relationship was investigated based on magnetic resonance imaging data and cognitive evaluations performed on participants of the Mexican Parkinson's Research Network. Left and right hippocampal volume was estimated in 48 patients with Parkinson's disease (21 females, mean age = 64.2 ± 11.6 years, symptom onset = 6.2, ± 4.1 years). The diagnosis of PD was made by a neurologist specializing in Movement Disorders (YMP). Magnetic resonance imaging was acquired in 3T equipment, including a high anatomical contrast image (T1) and high resolution (1x1x1 mm3). Cognitive assessment included the Montreal Cognitive Assessment (MoCA). Hippocampal volume was estimated with the assemblynet tool, in order to control for variability in global brain size, volume was expressed as a percentage of intracranial volume (% ICV, for Intracranial volume). The asymmetry of the left hippocampus with respect to the right hippocampus was also estimated by calculating the difference in percentage between the right hippocampal volume and the left hippocampal volume and divided by the total (bilateral) volume. A positive skewness indicates that the right hippocampal volume is larger, whereas a negative skewness implies larger left hippocampal volume. A general linear model was used to model the MoCA score based on hippocampal volume, including age, sex, and years of study as covariates. The analysis identified that left hippocampal volume significantly predicts MoCA score ($B = 3.83$, $SE = 1.86$, $p = 0.04$), whereas right hippocampal volume is not a significant predictor of MoCA score. Consequently, hippocampal asymmetry is also a significant predictor ($B = -0.21$, $SE = 0.07$, $p = 0.008$), reflecting that lower left hippocampal volume relative to right hippocampal volume results in lower MoCA score.

Disclosures: J. Esquivias: None. **C. Ponce Fernández:** None. **S. Alcauter:** None.

Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.05/D19

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG011230

Title: Executive Spectrum Abilities are Sensitive to Age-related White Matter Hyperintensity Progression: A Multi-Occasion Longitudinal Study

Authors: *J. LIU[1], C. DAHLE[2], A. M. DAUGHERTY[2,3], N. RAZ[1,4];
[1]Stony Brook Univ., Stony Brook, NY; [2]Inst. of Gerontology, [3]Dept. of Psychology, Wayne State Univ., Detroit, MI; [4]Max Planck Inst. for Human Develop., Berlin, Germany

Abstract: White matter hyperintensities (WMH) are common in older people and may augur cognitive decline. They reflect many pathological processes and are associated with cardiovascular and metabolic dysfunction. Yet, their implications for functional and cognitive change remain unclear, mainly due to the scarcity of longitudinal evidence. In 66 participants of the Detroit Aging Brain Study, aged 50-77 at baseline, we followed up WMH progression over 4 occasions spanning 7 years, while assessing changes in cardio-metabolic risk factors and cognition. We estimated WMH volume from T_2-weighted FLAIR images at 3 Tesla via automatic segmentation with longitudinal co-registration. The total WMH volume increased significantly over 7 years, and the change accelerated in late life across nearly all regions, most prominently in the frontal periventricular area. Higher baseline WMH volume correlated with greater progression across all regions. Men had greater WMH burden than women, independent of age, but did not differ in progression rate. Importantly, faster total WMH increases were coupled with steeper declines in perceptual speed and fluid cognitive ability. The perceptual speed decline was associated with an increase in parietal WMH, whereas a decrease in fluid cognitive abilities was coupled with progression of frontoparietal WMH. Notably, lower perceptual speed and fluid cognitive abilities at baseline correlated with faster WMH progression across the brain, especially in the parietal regions and deep white matter, indicating a bi-directional relationship between the state of the white matter and cognition, and signaling possible individual differences in brain maintenance. The elevation in systolic blood pressure was associated with faster total WMH progression. A higher baseline systolic blood pressure was associated with faster WMH accumulation in the temporal lobe. Thus, we found that not only does WMH volume increase over time in typical aging, but the change is also linked to declines in perceptual speed and fluid abilities - the quintessential age-sensitive skills.

Disclosures: J. Liu: None. **C. Dahle:** None. **A.M. Daugherty:** None. **N. Raz:** None.

Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

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Program #/Poster #: PSTR063.06/D20

Topic: C.01. Brain Wellness and Aging

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Title: Multilevel analysis of age-related functional and synaptic changes in the mouse brain

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Abstract: Aging is a complex, multifactorial process characterized by a gradual decline in brain function and plasticity, serving as the primary risk factor for the development of neurodegenerative disorders. Despite extensive research, the biological mechanisms underlying brain aging remain inadequately defined, particularly concerning region-specific vulnerability and sex-related differences. To address these gaps, we conducted a comprehensive multilevel analysis integrating behavioral, electrophysiological, histological, and molecular approaches to identify region- and sex-specific hallmarks of brain aging and to evaluate a potential therapeutic strategy targeting age-associated decline. We examined male and female C57BL/6 mice at 4, 14, and 24 months of age and intranasal administration of extracellular vesicles derived from human bone-marrow mesenchymal stem cells (hMSC-EVs; 1 dose/week for 4 months) to 20-month-old mice as a brain rejuvenation treatment, while age-matched controls received a vehicle. The Novel Object Recognition and Object Location tests revealed age-related impairments in cognitive domains, such as spatial and recognition memory, while motor deficits were observed in the Open Field and Grip Strength tests. Functional decline was accompanied by region-specific changes in dendritic spine density, as demonstrated by Golgi-Cox staining. Notably, we observed a reduction in spine density in the hippocampus and prefrontal cortex (PFC) and an unexpected increase in the motor cortex (M1), possibly reflecting compensatory remodeling of impaired cortical circuits. Immunofluorescence analyses indicated a reduction in parvalbumin-positive (PV^+) interneurons in the hippocampus and increased perineuronal net expression around PV^+ cells in M1 and PFC of older animals compared to the young group, suggesting region-specific adaptations of inhibitory networks. Western blot analyses on synaptosomal fractions from M1 revealed reduced expression of synaptic proteins, such as PSD95, synapsin, and SNAP25, and altered phosphorylation of key plasticity-related signaling molecules, indicating synaptic impairment at the molecular level. Importantly, preliminary results suggest that hMSC-EVs treatment partially ameliorated both cognitive and motor deficits in aged mice, supporting its potential as a non-invasive therapeutic approach to mitigate age-dependent functional decline. In conclusion, our study underscores the complexity of brain aging, revealing region- and sex-dependent alterations at multiple biological levels and suggests that hMSC-EVs could represent a promising avenue for therapeutic intervention.

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Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.07/D21

Topic: C.01. Brain Wellness and Aging

Title: Mct may reduce the severity of delirium and lower mortality

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Abstract: Introduction Delirium is an acute syndrome characterized by disturbances in consciousness, attention, and perception, often triggered by physical abnormalities such as infections, surgical invasions, cerebrovascular disorders, or electrolyte imbalances. It has been reported that delirium increases the risks of mortality, prolonged hospital stays, and long-term cognitive decline. Despite its clinical significance, effective methods for preventing and treating delirium have not yet been established, highlighting the need for novel therapeutic strategies. Inflammation and oxidative stress in the brain are thought to play central roles in the pathophysiology of delirium. β-hydroxybutyrate (BHB), a type of ketone body, has been reported to exhibit anti-inflammatory and antioxidant effects. Additionally, orally administered medium-chain triglycerides (MCTs) are rapidly converted to BHB in the liver. Based on this evidence, we hypothesized that MCT may have delirium-preventive effects. Method Male C57BL/6 mice, approximately 50 weeks old, implanted head-mounted were used. To make delirium mice model , LPS (0.5-1.0 mg/kg) was administered intraperitoneally. MCT oil or long-chain triglyceride (LCT) oil were administered via a gastric tube prior to LPS administration. Electroencephalograms (EEG) were recorded before and after LPS administration. EEG data were converted to BSEEG scores (Yamanashi et al., J Psychiatr Res. 2021), which quantify slow-wave activity and have been shown to be useful in assessing delirium severity in humans (Yamanashi et al, Br J Psychiatry 2021). Results Oral administration of MCT oil suppressed the increase in BSEEG scores in mice model of LPS-induced delirium . In the LCT-administered group, a certain proportion of mice exhibited mortality following LPS administration, whereas the mortality rate was lower in the MCT-administered group. Conclusion These findings suggest that oral intake of MCT oil may reduce the severity of delirium and improve outcomes. All procedures were conducted in accordance with the ethical guidelines of the Tottori University Animal Care and Use Committee and complied with institutional regulations.

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Poster

PSTR063: Aging: Behavior and Cognitive Disorders

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Program #/Poster #: PSTR063.08/D22

Topic: C.01. Brain Wellness and Aging

Support: Secihti 800824
Secihti 626561
Secihti 1079765
Secihti 1241800
Secihti 2060348

Title: Impact of High-Fat Diet on Cognitive and Motor Function in Young and Middle-Aged Mice.

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Abstract: Obesity is a chronic metabolic disorder characterized by excessive body fat accumulation and is linked to an increased risk of cardiovascular disease, type 2 diabetes, and cancer. Beyond its systemic effects, obesity has also been associated with impaired brain function and a higher incidence of cognitive decline. Aging, on the other hand, entails progressive biological changes in the brain, including volume reduction in memory-related regions like the hippocampus and the prefrontal cortex. Obesity has been suggested to accelerate biological aging, yet the degree to which these processes interact to influence cognitive and motor performance remains unclear. In this study, we investigated the impact of a high-fat diet on cognitive and motor function in young (3-month-old) and middle-aged (12-16 months old) C57BL/6 mice. The animals underwent behavioral assessments, including the novel object recognition test (NORT) and the nest-building test. Our results show that both age groups experienced memory impairment following exposure to a high-fat diet, as indicated by reduced performance in the NORT. However, only young mice exhibited significant deficits in nest-building behavior, suggesting an age-dependent vulnerability in motor functions. These findings indicate that HFD-induced cognitive impairments occur regardless of age, while motor

impairments are more pronounced in young mice. This study highlights the significance of age as a factor in obesity-related neurobehavioral outcomes, thereby contributing to our understanding of how diet and aging impact brain function.

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Poster

PSTR063: Aging: Behavior and Cognitive Disorders

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Program #/Poster #: PSTR063.09/D23

Topic: C.01. Brain Wellness and Aging

Support: P51OD011107 (CNPRC base grant; PI: Atkinson)
P51OD011107-62S3 (PI: Erin Kinnally)
R21AG081998 (PI: Erin Kinnally)

Title: Behavioral and Physiological Indicators of Mortality in Aging Rhesus Macaques

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Abstract: Nonhuman primates (NHPs) provide a powerful translational model for identifying behavioral and physiological patterns that precede mortality in aging populations. Subtle indicators of decline can improve clinical insight and welfare in both NHPs and humans. We analyzed data from 171 rhesus macaques who completed the Geriatric Biobehavioral Assessment (GBA) at the California National Primate Research Center. The GBA is a 25-hour standardized protocol assessing social, motor, emotional, and cognitive functions in rhesus macaques aged 15 to 25 years. We examined biobehavioral predictors of 5-year survival among aging animals. Animals were grouped as alive (n=123) or deceased (n=48) based on confirmed death records post-GBA. Two-tailed t-tests compared groups across behavioral and physiological domains. Animals that died within five years of GBA assessment exhibited differences in oxygen-related biomarkers, including O_2 content ($p = 0.0003$), O_2 saturation ($p = 0.0001$), and pO_2 ($p = 0.0028$), along with lower pCO_2 at the trend level($p = 0.0942$). Sodium was lower ($p = 0.0353$), and CRP trended higher ($p = 0.0868$), suggesting higher inflammatory burden in short-lived animals. Behaviorally, short-lived animals showed less emotional expression (fear grimacing ($p = 0.0683$), self-clasping ($p = 0.0024$), tooth grinding ($p = 0.0983$)and cage shaking ($p = 0.0057$)), with trends toward reduced drinking, hanging, and sexual display. In contrast, they spent more time eating ($p = 0.0413$), which may reflect compensation for metabolic inefficiency. This

pattern of lower stress expression, reduced activity, and increased food intake may connect physiological and behavioral signs of decline.These findings suggest that reduced stress behaviors and activity, paired with increased food intake, may reflect more than emotional well-being but rather physiological exhaustion. Though outwardly benign, these behaviors may signal a compensatory state preceding clinical decline. Elevated oxygen and inflammatory markers support this interpretation and highlight the importance of integrating behavioral and physiological monitoring in aging research.

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Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.10/D24

Topic: C.01. Brain Wellness and Aging

Title: Reevaluation of Age-Related Behavioral Phenotypes in the SAMP8 Mouse Model of Senescence

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Abstract: The senescence-accelerated mouse-prone (SAMP) line was first identified at Kyoto University in the early 1970s, when investigators observed that offspring from an inbred AKR/J colony exhibited signs of accelerated aging. These offspring were selectively bred to produce several SAMP lines, including the SAMP8 line, which models a range of age-related central nervous system dysfunctions. SAMP8 mice show early-onset characteristics of senescence, including cognitive impairment and deficits in motor coordination and endurance. As the SAMP8 line has existed for nearly five decades, concerns have emerged about potential genetic drift leading to a gradual loss of phenotypes originally associated with the model. To assess whether the SAMP8 mice at Inotiv continue to exhibit canonical phenotypes reported in the literature, we evaluated male and female SAMP8 and senescence-accelerated mouse-resistant (SAMR1) mice—the phenotypically normal control line—on a battery of cognitive and motor tasks at 25-37 weeks of age. Behavioral assays included the Morris water maze, Y-maze, fear conditioning, open field, elevated plus maze, rotarod, grip strength, and wire hanging task. Relative to SAMR1 mice, SAMP8 mice showed deficits in spatial learning in the Morris water maze, hyperactivity in the open field, decreased anxiety-like behavior in the elevated plus maze, impaired performance on the rotarod, reduced grip strength, and lower endurance in the wire hang task. These results align with published studies characterizing SAMP8 mice. However, in contrast to prior findings, SAMP8 mice in our study did not differ from SAMR1 mice in Y-maze alternation or contextual fear conditioning, suggesting that some phenotypic drift may have occurred. Collectively, these findings indicate that the SAMP8 line retains many core features of

senescence-associated cognitive and motor decline, supporting its continued utility in aging research.

Disclosures: L.E. Hood: A. Employment/Salary (full or part-time): Inotiv. 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.; Inotiv. **C.W. Bird:** A. Employment/Salary (full or part-time): Inotiv. 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.; Inotiv. **J.D. Shusterman:** A. Employment/Salary (full or part-time): Inotiv. 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.; Inotiv. **C.M. Butt:** A. Employment/Salary (full or part-time): Inotiv. 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.; Inotiv.

Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.11/D25

Topic: C.01. Brain Wellness and Aging

Support: 1R01AG07544
1RF1AG074256-01A1

Title: Psilocybin Alleviates Chronic Stress-Mediated Cognitive and Mood Impairments

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Abstract: Major Depressive Disorder (MDD) affects over 280 million individuals globally and remains a leading cause of disability worldwide. Approximately 30-40% of patients with MDD exhibit treatment-resistant depression and fail to respond to standard pharmacological therapies.

Psilocybin (PS), a psychedelic compound and 5-hydroxy tryptamine 2A (5-HT2A) receptor agonist, has been effective in promoting rapid and sustained antidepressant effects in early-phase clinical trials. PS can modulate large-scale neural networks involved in emotional regulation and executive function, such as the default mode network and hippocampal-prefrontal circuitry. To investigate the efficacy of PS in preventing chronic stress-mediated MDD, we developed a rat model of chronic stress that exhibits significant cognitive and mood deficits akin to that observed in MDD. Wistar rats were subjected to 6 hours of restraint stress per day for 21 consecutive days. Psilocybin (1 mg/kg, oral) was administered five times, with three doses delivered on chronic stress days 7, 14 and 21 and three doses thereafter on post-stress days 7 and 14. Following the completion of the treatment regimen, animals were assessed using a comprehensive battery of neurobehavioral tests designed to evaluate both hippocampus-dependent cognitive function and core affective symptoms relevant to MDD, including behavioral despair, anhedonia, and anxiety-like behavior. PS treatment significantly improved hippocampus-dependent cognitive function in an object location test (OLT) and recognition memory function in a novel object recognition test (NORT), compared to chronically stressed rats receiving vehicle treatment, which exhibited impairments in these tasks. In the Forced Swim Test (FST), PS-treated chronically stressed rats spent reduced amounts of time in immobility compared to the vehicle-treated chronically stressed rats, suggesting reduced depressive-like behavior. Similarly, improvements were observed in the Novelty Suppressed Feeding Test and the Sucrose Preference Test, suggesting a reduction in anxiety-like behavior and anhedonia. Brain tissue analyses are currently in progress to understand the mechanisms by which PS maintains better cognitive and mood function in chronically stressed rats. These findings suggest that psilocybin has the potential to prevent chronic stress-mediated MDD. Funding: This work is supported by grants from the Steadman Philippon Research Institute and National Institute on Aging (1R01AG075440 and 1RF1AG074256-01A1) to A.K.S.

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Poster

PSTR063: Aging: Behavior and Cognitive Disorders

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR063.12/D26

Topic: C.01. Brain Wellness and Aging

Title: Ketamine improves neurocognition by improving synaptoysin expression while mitigating astrogliosis in animal model of depressive disorder

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Abstract: Depression is a mood disorder attributed to psychological stress and it is one of the leading cause of death global, hence of public health concern since the common drug (Fluoxetine) administered have been reported to have a delayed response time. Hence, the need to identify a fast-acting drug. Ketamine, an N-Methyl D-Aspartate (NMDA) receptor antagonist, has been reviewed to have potential as a fast-acting neurocognitive drug; however, its mechanism of action is not fully ascertained. This study is aimed at ascertaining the interplay between synaptophysin and astrocytes expression in fluoxetine and ketamine-treated models of depression and establishing its role in synaptic connection, neuroinflammatory response of astrocytes and changes in immobility time a neurobehavioral paradigms for predictive validation of antidepressant drugs. The Forced Swim Test (FST) model was adopted. 20 mg/kg (IV) ketamine and 20 mg/kg oral fluoxetine were given. 30 adult male Wistar rats were divided into six groups (n = 5): A = control, B = FST, C = ketamine, D = fluoxetine, E = FST + ketamine, and F = FST + fluoxetine. Statistical significance was set at $p < 0.05$ using the Tukey post-hoc test. This study showed that the ketamine-treated and fluoxetine-treated FST groups significantly improved anhedonia, reduced immobility time, and improved synaptophysin expression while reducing astrocytic proliferation when compared to the FST group at $p<0.05$. However, when comparing the two drugs, ketamine had a statistically significant improvement over fluoxetine in reducing anhedonia, immobility time, astrocytic proliferation while increasing the expression of synpatophysin in the prefrontal-cerebellar cortices. Ketamine's rapid antidepressant potential mechanism is attributed to its anti-inflammatory potential of reducing astrogliosis, which improves synaptic connections and synaptogenesis, resulting in improved neurocognitive functions of neurons in the prefrontal-cerebellar cortex that averts behavioral despair in the FST model in depressive animals.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.01/D27

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

Support: U19AG060909

Title: An integrated, multiregional, and multimodal cell atlas of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is clinically characterized by progressive cognitive decline associated with stereotyped accumulation of amyloid-beta (Aβ) plaques and hyperphosphorylated Tau (pTau) tangles across brain regions. While histopathology has revealed patterns of regional involvement, the molecular and cellular events that accompany and potentially drive this progression remain incompletely understood. The Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD) applied single nucleus RNA sequencing (RNAseq), ATAC-seq, and Multiome profiling to over 7 million high-quality nuclei from 10 brain regions—spanning medial and lateral entorhinal cortices, hippocampus, multiple temporal and frontal cortical areas, and primary visual cortex—sampled from the same cohort of 84 aged human donors across the AD spectrum (3 regions from all donors, 7 from those without severe co-morbidities). Cell-types were predicted for nuclei by mapping to an expanded BRAIN initiative cell-type taxonomy, which included AD-associated non-neuronal states and ~70 brain region-specific neuronal types. These datasets were paired with regional quantitative measurements of Aβ (6e10), pTau (AT8), and other protein pathologies, as well as cellular stains for neurons, microglia, and astrocytes. We inferred two distinct protein pathology accumulation patterns across brain regions: neocortical areas accumulated Aβ prior to pTau, whereas the hippocampus and entorhinal cortex had early and, in some cases, substantial pTau burden independent of Aβ. Among neocortical regions, accumulation of AT8 beyond the temporal medial lobe was strongly associated with dementia. In analyzing cell-type abundance differences associated with higher levels of pTau pathology, we identified shared motifs of selective neuronal vulnerability consistent with our previous observations from the middle temporal gyrus. We also observed lower abundances in specific, regionally specialized neuron subtypes in the hippocampus, entorhinal cortex, and visual cortex. Our multimodal, multi-region single-cell atlas revealed shared and region-specific patterns of cellular vulnerability in AD. These cell-types, particularly those commonly affected in distinct neural circuits, could serve as candidate therapeutic targets and biomarkers.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.02/D28

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

Support: NIH Grant UM1MH130981

Title: A Comprehensive Transcriptomic Map of the Human Medial Temporal Lobe

Authors: *A. D. GARCIA[1], R. FERRER[1], D. YUAN[1], K. J. TRAVAGLINI[1], J. T. MAHONEY[1], N. JOHANSEN[1], T. CASPER[1], A. CHAKKA[3], N. DEE[1], J. ARIZA TORRES[4], J. GOLDY[5], S. BARTA[4], D. L. JONES[1], L. KRUSE[1], A. TORKELSON[2], B. P. LEVI[6], K. A. SMITH[2], C. D. KEENE[9], Z. YAO[1], T. BAKKEN[7], H. ZENG[1], E. LEIN[6], R. D. HODGE[8]; [2]Mol. Biol., [1]Allen Inst. for Brain Sci., Seattle, WA; [3]Mol. Biol., [4]Histology, Allen Inst., Seattle, WA; [5]BiCore, [6]Human Cell Types, [8]Cell Types Program, [7]Allen Inst. For Brain Sci., Seattle, WA; [9]Pathology, Univ. of Washington, Seattle, WA

Abstract: The structures within the medial temporal lobe (MTL), including the amygdala, hippocampal formation, and several neocortical association areas such as the perirhinal cortex, facilitate myriad critical cognitive functions including emotional regulation, learning, and memory. Moreover, several of these components are among the first and most critically debilitated regions impacted during the development of neuropathological dementias such as Alzheimer’s Disease. Designing cell-type-specific therapeutics to preserve these areas and defend against these debilitating neuropathologies necessitates an understanding of the complete diversity of cell types within the MTL. However, there is currently no comprehensive atlas of cellular diversity comprising these regions of the human brain. In the present study, we captured 1.7 million nuclei across ten human post-mortem donors using single-nucleus RNA sequencing to comprehensively define cell types across the full anatomical extent of the human MTL. We identify putative excitatory types specific to the amygdala, entorhinal cortex, hippocampal sub-regions, and the extended subicular complex. Furthermore, by sampling at a fine resolution across the anterior-posterior axis, we identified gradients in gene expression that disambiguate anatomically distinct positions across the long axis of the hippocampal formation. Finally, we leverage gene set enrichment analysis to infer relationships between putative gene expression patterns and unique properties of the primate hippocampus relative to that of the rodent (i.e. cross-species network physiology differences). Taken together, this work represents a major effort to holistically profile the medial temporal lobe and understand its constituent cellular populations at multiple anatomical scales.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.03/D29

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

Support: 2021R1A2C3007164
HU20C0066
2022M3E5E8016325

Title: Cell-specific Alterations in the Thalamus of an Alzheimer's Disease Mouse Model

Authors: *G. HA[1], H.-E. PARK[2], Y. LEE[1], D. LEE[1], C. SOHN[2], E. CHEONG[1];
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Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by brain pathologies, such as the accumulation of amyloid plaques and neurofibrillary tangles, leading to neuronal damage and cognitive decline. Many studies have focused on memory-related brain regions, such as the medial prefrontal cortex and the hippocampus. However, our histological screening of AD mice in 3- to 12-month-old age revealed early and progressive pathophysiological changes in the thalamus, including the accumulation of amyloid plaque and reactive astrogliosis. To investigate the cell type-specific alterations in the thalamus during AD progression, we conducted single nucleus RNA sequencing using the thalamus of AD and wild type mice. This analysis identified transcriptionally distinct subpopulations in major brain cell types, including astrocyte and microglia, and revealed disease-associated genes and dysregulated molecular pathways in a cell type-specific manner. Our single nucleus transcriptomic resource previously underexplored thalamic vulnerability in Alzheimer’s disease and provides molecular insights into cell-type-specific contributions to disease pathogenesis.

Disclosures: G. Ha: None. **H. Park:** None. **Y. Lee:** None. **D. Lee:** None. **C. Sohn:** None. **E. Cheong:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.04/D30

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

Support: Chan Zuckerberg Initiative 2019-199150
Cure Alzheimer's Fund 2023-Cobos

Title: A Multi-omics Approach to Characterize Neurofibrillary Tangle-Bearing Neurons in the Hippocampus and Neocortex of Alzheimer's Disease and Primary Age-Related Tauopathy

Authors: *D. PARK[1], J. FORES MARTOS[2], A. SANKARAESWARAN[2], J. SANZ-ROS[2], S. IVEZICH[2], M. OTERO-GARCIA[3], T. RICHARDSON[4], J. M. WALKER[4,5], J. F. CRARY[4,6], I. COBOS[2];
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Abstract: Hyperphosphorylated tau (p-tau) in neurofibrillary tangles (NFTs) and amyloid-beta (Aβ) plaques are key pathological hallmarks of Alzheimer's disease (AD), yet their molecular interplay during disease progression remains unclear. By contrast, primary age-related tauopathy (PART) exhibits tau aggregates biochemically identical to those in AD but lacks Aβ plaques; it typically progresses slowly and remains confined to limbic regions. Here, we generated single-cell multi-omics data from the human hippocampus and prefrontal cortex (BA9) to characterize the molecular signatures of NFT-bearing neurons in AD and PART across Braak stages. We employed a multi-omics approach that integrated whole transcriptomes (10x Genomics), 325 proteins (Proteintech MultiPro Human Discovery Panel), and 4 p-tau proteins on well-preserved, fixed NFT-bearing somas. Custom DNA-barcoded p-tau antibodies targeting early (pT181, pT217, pT231) and late (pS396) tau aggregates allowed quantification of tau pathology progression, from diffuse, non-fibrillar p-tau accumulation (pre-tangles) to fully formed NFTs. This approach enabled high-resolution profiling of 400,000 hippocampal somas (23 donors) and 710,000 neocortical somas (22 donors) across the spectrum of AD and PART progression, and age-matched healthy controls. To further investigate microenvironmental factors influencing tau pathology in AD (Aβ-positive environment) and PART (Aβ-negative environment), we utilized spatial transcriptomics (10x Xenium) followed by post-Xenium staining for Aβ plaques and p-tau. Our datasets provide insights into how cell-type-specific vulnerabilities, along with Aβ and glial-neuronal interactions, contribute to the progression of tau pathology in neurons across different brain regions.

Disclosures: D. Park: None. **J. Fores Martos:** None. **A. Sankaraeswaran:** None. **J. Sanz-Ros:** None. **S. Ivezich:** None. **M. Otero-Garcia:** None. **T. Richardson:** None. **J.M. Walker:** None. **J.F. Crary:** None. **I. Cobos:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.05/D31

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

Support: NIH R01 AG071859
NIH R01 AG073826

Title: The Retinoic Acid Hypothesis in Alzheimer's Disease: Transcriptomic Profiling Reveals Cell Clusters in the Dentate Gyrus Implicated in Regulation of Excitation/Inhibition Balance

Authors: *P. E. LIE[1,2,3,4,5], A. BAKER[1,2,3,4], S. SMITH[1,2,5], J. STRICKLAND[1,2], S. SKAWRATANANOND[1,2], B. R. KISBY[1,2,3,6], S. SHANMUGAM[1,2,6], A. HINDLE[1,2], M. TELIAS[7,8], I. PONOMAREV[1,2,3,9,6], J. J. LAWRENCE[1,2,3,9,10];
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Abstract: Although the accumulation of amyloid-β is implicated as a primary mechanism of Alzheimer's disease (AD), effective prevention and treatment modalities have yet to be elucidated. Hyperexcitability and impaired excitatory/inhibitory (E/I) balance are observed in the hippocampal dentate gyrus early in AD, yet the underlying molecular mechanisms remain unclear. Converging evidence also suggests that all-trans retinoic acid (ATRA), the biologically active metabolite of Vitamin A (VA), directs amyloid peptide protein processing to the non-amyloidogenic pathway. We tested the hypothesis that dietary VA levels regulate cognitive performance, AD pathogenesis, and transcriptomic profiles in hybrid RARE-lacZ/J20 AD mouse models. RARE-lacZ/J20 mice were weaned onto standard (4 IU/g) or VA-deficient (0.4 IU/g) diets. Water T-Maze (WTM) was implemented to test simple discrimination (SD) and reversal learning (RL) at P125. Dietary treatment and genotype significantly altered distance traveled during SD, while genotype alone significantly affected latency to platform during RL ($n=96$, $p<0.05$, mixed-effects model). Additionally, RARE-lacZ/J20 mice on a VA-deficient diet made more errors locating the platform than WT mice on a control diet during the simple discrimination phase ($n=86$, $p<0.05$, two-way ANOVA with Tukey's post-hoc analysis). Consistent with Yeo et al. 2024, a subset of mature hippocampal dentate gyrus granule cells (DGGCs) expressed LacZ. Transcriptomic analysis (Parse mini-kit, 20k reads/nuclei) of DG cell types in RARE-LacZ/J20 mice found distinct clusters of DGGCs, interneuron subclasses, and glial populations. Within DGGCs, several differentially expressed genes associated with E/I balance were altered in J20 compared to WT mice, including GRIA2, KCNC4, and GABRA5. AD-induced downregulation of KCNC4 and upregulation of GABRA5, GRIA2 may possibly contribute to E/I imbalance and functional dysregulation. Comparison on Enrichr with reference to RF-LOF GEO found that NRF2 is upregulated in the J20 genotype ($p<0.001$), supporting the hypothesis that oxidative stress precedes AD. Finally, two novel GABAergic clusters were retinoic acid receptor β/Reelin (RARB/RELN) positive. This finding supports the hypothesis that ATRA depletion in AD impairs inhibition, disrupting E/I balance and sparse coding in the DG. One of the two RARB/RELN clusters expressed DRD1, while the other exclusively expressed DRD2, suggesting complex dopamine modulation of novelty detection in DG. Future studies will further investigate the role of ATRA in regulating DGGC excitability and E/I imbalance in AD.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.06/D32

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

Support: The Fisher Center for Alzheimer's Research Foundation
CHDI Foundation

Title: Cell Type-Specific Deep Molecular Profiling for Cross-Disease Comparisons of Neocortical Alterations in Huntington's and Alzheimer's Disease

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Abstract: Neurodegenerative diseases, such as Alzheimer's (AD) and Huntington's disease (HD) affect distinct cell types of the human neocortex. In HD, upper layer pyramidal neurons of layers 2/3 are relatively spared early in the course of the disease, while in mouse models of AD, these cells have been shown to exhibit hyperexcitability, and their involvement in AD neuropathology has been suggested. In contrast, deep layer cortical neurons in HD exhibit dysregulation of hundreds of genes, and L5a pyramidal neurons are lost early in the disease. In AD, these cells also show signs of altered synaptic processing. Moreover, alterations in the affected cell types can trigger reactive downstream changes in neighboring and connected cells. As a result, certain neocortical regions are more heavily affected by HD compared to AD, and vice versa. Due to this complex and cell type-specific interplay, region- and cell type-specific studies are imperative to understand the intricate disease mechanisms in human neurodegeneration. Disentangling cell-autonomous from reactive events is essential to ultimately uncover novel and causal therapeutic targets. We applied serial Fluorescence Activated Nuclei Sorting (sFANS) to sort cell nuclei from fresh frozen postmortem human neocortical brain samples. The neocortical cell types that can be isolated using sFANS include excitatory neurons of layers 2/3, 4, 5a, 6a, and 6b, Betz cells, and Von Economo neurons, inhibitory neuron populations, including VIP, RELN, LAMP5, and PVALB-expressing neurons, and the four glial cell types: astrocytes, microglia, oligodendrocytes and oligodendrocyte precursors. Nuclei from these populations are sorted into separate tubes for cell type-specific downstream processing. For this project, we used postmortem fresh frozen cortical brain samples of early-stage AD and HD, as well as control (NC) donors. Sorted nuclei were processed for RNA sequencing, and assays for transposase accessible chromatin (ATAC) were performed for ATAC sequencing. RNAseq data were analyzed for cell type-specific differential gene expression using DESeq2, and results were filtered for genes with accessible promotors in each cell type using the corresponding

ATACseq datasets. Our analyses reveal both cell type- and disease-specific dysregulation, as well as changes that are shared between conditions that are likely reactive and downstream of the initiating pathophysiological event. In conclusion, sFANS enables generation of robust, deep, and highly reproducible molecular profiling data from the major neocortical cell types, making it highly suitable for cell type-specific studies of neurodegeneration.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

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Program #/Poster #: PSTR064.07/D33

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

Support: NIH Grant R01AG081374

Title: Cell type-specific functional impacts of lipid droplets in Alzheimer's Disease iPSC-derived neuron/astrocyte co-cultures

Authors: *C. THAPA[1,5], H. ZHANG[1], S. ZHANG[1,5], A. KOZLOVA[1], W. G. WOOD[1], B. JAMISON[1], B. A. CHASE[2], R. FRIGERIO[3], K. MARKOPOULOU[4], Z. P. PANG[6], A. SANDERS[1,5], J. DUAN[1,5];
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Abstract: The dysregulation of lipid droplets (LD) is increasingly appreciated in Alzheimer's disease (AD), and is influenced by AD genetic risk factors. Despite extensive studies of LD in glial cells, the role of neuronal LD in AD, especially in the context of neuron/glia interaction, remains poorly understood. In this study, we characterize cell-type-specific morphometric and molecular impacts of LDs across neural populations using co-culture models (excitatory, inhibitory and astrocytes) derived from a cohort of AD-specific (42 individuals) and age-matched control (15 individuals) iPSC lines. To dissect the molecular underpinnings of AD compared to control conditions, we profiled single-nucleus transcriptomes and chromatin accessibility using single-nucleus multiomic sequencing (RNA/ATAC-seq). AD cells (compared to control) showed a global upregulation of stress and metabolic pathways alongside downregulation of genes related to synaptic signaling, function, neuron projection arborization and energy production. Preliminary morphometric data displayed a decrease in neurite outgrowth, synaptic puncta count and puncta area in AD excitatory neurons. These results suggest impaired neuronal communication and a potential shift toward a reactive, survival-focused state. Next, to modulate LD accumulation, we treated the neuron/glia co-culture of the

same donor line with Triascin C (TC), an inhibitor of long-chain acyl-CoA synthetases (ACSL) that blocks LD formation. TC treatment led to a significant reduction of LD count per cell across all cell types and a decreased LD area per cell in excitatory neurons, with effects observed irrespective of disease status. Morphometric data further revealed that TC treatment significantly upregulated puncta area but reduced neurite outgrowth, number of processes and branches in AD excitatory neurons. Multiomic data showed that although all three cell types exhibited upregulation of sterol and cholesterol biosynthesis and downregulation of dendrite morphogenesis associated genes, astrocytes displayed the most pronounced transcriptional and chromatin accessibility changes, underscoring their central role in the cellular response to lipid droplet dynamics and AD-related pathology. Our study suggested that modulating lipid metabolism may alter both structural and functional properties of neurons, highlighting the investigation of lipid metabolic pathways as potential therapeutic targets for mitigating neurodegeneration in AD.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.08/D34

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

Support: NIH Grant AG064664 (to QW)

Title: Gene prioritization analysis reveals common molecular signatures between Alzheimer’s Disease and Heart Failure

Authors: *Y. GONZALEZ-GIRALDO[1], K. JIAO[2], Q. WANG[3];
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Abstract: Alzheimer’s Disease (AD) and cardiovascular diseases (CVD) share several risk factors, such as genetic variants and clinical conditions. Epidemiological studies have demonstrated that many CVDs, including the early onset of heart failure (HF), increase the risk of developing AD and other dementias. In this study, we aimed to identify common molecular signatures between AD and HF through a comprehensive bioinformatics analysis. We used a gene prioritization analysis considering mRNA genes and regulatory molecules (microRNAs) previously investigated in AD and HF studies. Genes were prioritized based on different approaches such as meta-analyses, gene overlapping, interaction network and enrichment analyses. Finally, to detect the potential effect of these common signatures in specific cell types,

the expression of prioritized genes was explored in brain and heart tissues using single-cell transcriptomic datasets. Our miRNA meta-analyses involved in total of 349 cases and 351 controls for AD, and for HF, 89 cases and 74 controls. Only three common miRNAs were detected with consistent expression in brain and heart tissue, but no shared signatures were detected in blood samples. These miRNAs regulate several common genes, which are associated with the regulation of histone modifying activity, primary metabolic processes, among others. By comparing genes identified in previous transcriptomic meta-analyses, only 4 dysregulated genes were observed in samples from both diseases. In the network analysis, some of these signatures were identified as hub genes, suggesting an important regulatory role. These preliminary findings extend the knowledge of commonalities between AD and heart failure, providing a list of candidate target genes and pathways that could be useful for further studies focused on exploring the link between HF and AD or for identifying interventions to treat or prevent both pathologies.

Disclosures: Y. Gonzalez-Giraldo: None. **K. Jiao:** None. **Q. Wang:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.09/D35

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

Support: COBRE 5P20GM121310-07
National Institute of Aging
Office of the Director
National Institute of Health
Wyoming Sensory Biology Center

Title: Spatial and single-cell transcriptomic landscape changes in a mouse model of Alzheimer's Disease

Authors: *M. SCHULTZ[1], Q.-Q. SUN[2];
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Abstract: Alzheimer’s Disease (AD) is a progressive neurodegenerative dementia claiming the life of one person worldwide every twenty seconds. Conventional research methods often focus narrowly on single etiological factors, limiting a comprehensive understanding of multifaceted pathophysiology. To understand complex cellular interactions, high-resolution molecular profiling tools are needed to provide unbiased insights at the individual cell level. Using the 5xFAD mouse model, single-cell RNA sequencing (Chromium Fixed RNA Profiling) and high-definition spatial gene expression mapping (Visium HD) reveals cellular profiles, gene expression maps, and rare cell types uniquely associated with AD. Additionally, downstream analyses allow interpretation of biological processes, functions, and cellular components affected in AD progression. Specifically, Visium HD gene ontology (GO) analyses in 14-week-old

presymptomatic 5xFAD mice revealed that differentially expressed genes are enriched in pathways related to cell division in astrocytes and sterol biosynthesis in specific cortical layers (L2/3/5/6). Additionally, enrichment of GPCR signaling pathways was observed in the hypothalamus and pallidum. In parallel, scRNAseq gene ontology analyses in presymptomatic 5xFAD mice revealed enrichment of differentially expressed genes related to cell metabolism in glutamatergic neurons. In symptomatic 5xFAD mice, scRNAseq gene ontology analyses revealed enrichment of differentially expressed genes related to cellular growth and recovery, synaptic plasticity, and protein misfolding within astrocyte and glutamatergic neuron cell types. Collectively, these complementary profiling techniques provide a systems-level perspective on the multifactorial AD etiology, paving the way for development of novel biomarkers and therapeutic targets. Biological implications and their connection to AD etiology are discussed in greater detail.

Disclosures: M. Schultz: None. **Q. Sun:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

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Program #/Poster #: PSTR064.10/D36

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

Support: NIH Grant 5U24AG061340-07

Title: A multi-omic data ecosystem for Alzheimer’s research: infrastructure and impact of the AD Knowledge Portal

Authors: *J. SCANLAN, J. BECK, W. POEHLMAN, T. ADAMS, V. BAHAM, J. S. BRITTON, A. CAMPTON, A. KALLAHER, M. KLEIN, K. LEAL, Z. LEANZA, J. LUNDIN, J. MALENFANT, A. PENA, B. SALDANA, T. ZINTEL, S. AHMED, R. AYYALA, J. DRISCOLL, C. SUVER, S. VARMA, L. HEATH;
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Abstract: Background: The AD Knowledge Portal (adknowledgeportal.org) is an NIH-designated data repository and distribution site for multi-omic data from both human and model systems. This resource enables broad access to both raw and processed omics data, analytical methodology, and research tools generated by the Alzheimer’s Disease research community. The Portal was created and is maintained by Sage Bionetworks, and is funded by the National Institute on Aging (5U24AG061340-07).
Methods: The Portal hosts resources generated through 14 different NIH-funded research programs related to dementia and aging, including a Community Data Contribution Program (CDCP), which enables data sharing for research groups not affiliated with established AD Knowledge Portal consortia. Both Portal users and contributors span NIA-sponsored consortia as well as research groups supported by other NIH institutes, federal agencies, non-profit

foundations and for-profit organizations.
All Portal resources are available to approved researchers for General Research Use under either open or controlled access terms, depending on their level of sensitivity. Access to these resources is mediated through the Synapse (synapse.org) data sharing platform via a user-friendly front-end.
Results: Portal resources encompass ~850TB of data from over 11,000 individuals. This extensive collection includes next-generation sequencing, proteomics, metabolomics, imaging, and behavioral data from brain banks, longitudinal cohorts, cell lines, and animal model systems. Over the past two years, data contributions have included an increasing volume of multimodal single-cell omics data and other novel data types, amounting to more than 300TB currently available.
Additional resources available to users include well-characterized experimental mouse and marmoset models, publications, computational tools, processed data, analytical results, Target Enabling Resources, and summarized evidence for putative AD drug targets via integrated results explorers. Furthermore, Portal resources can be combined with externally-sourced research datasets via interoperability with several cloud-based analysis platforms. These integrations enable reuse of large datasets and contemporary data types by offering an alternative to direct download. In total, more than 15 PB of data from the Portal has been downloaded by more than 7,000 unique users since January 2022.
Conclusion: The AD Knowledge Portal continues to facilitate the translation of rich, high-throughput data into valuable scientific insights in the field of Alzheimer's Disease research through responsible open data sharing.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

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Program #/Poster #: PSTR064.11/D37

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

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NIH Grant K08AG065502
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NIH Grant R01AG088082
Alzheimer's Association Research Fellowship

Title: Neurons accumulate disease-specific somatic genomic changes across tau pathologic states in Alzheimer's disease

Authors: *K. S.-M. BROWN[1], D. SMIRNOV[2], G. DONG[1], D. J. SELKOE[3], M. FROSCH[4], D. H. OAKLEY[4], B. HYMAN[5], Y. HUANG[6], M. MILLER[2];
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Abstract: Tau deposition within neurons marks Alzheimer's disease (AD) neuropathology, suggesting that tau contributes to cellular dysfunction and death. While only a subset of neurons within affected brain regions exhibits tau pathology in AD, the etiology and pathological implications of this heterogeneity remain unclear. In AD, somatic mutations accumulate in neuronal genomes, with features that suggest deleterious effects on cellular function. Comparing the somatic genomic landscape of AD neurons with distinct tau states may reveal whether the accumulation of somatic mutations in AD are driven by disease pathology. To examine the relationship between tau and somatic mutation, we developed and applied a fluorescence-activated nuclear sorting method to isolate single neurons according to tau pathology. We then performed single-cell whole-genome sequencing on tau+, tau-, and tau-agnostic neurons from 13 individuals with AD, as well as neurons from 15 control individuals. We found that AD neurons, regardless of their tau status, exhibited an increased burden of somatic single-nucleotide variants (sSNVs) and insertions and deletions (sIndels). Mutational signature analyses implicate disease-associated oxidative damage as a contributor to sSNVs and reveal a novel disease-specific sIndel pattern characterized by two-basepair deletions, indicating shared mutagenic mechanisms in AD neurons across tau pathologic cell states. In contrast to these single-neuron findings, immunohistochemistry showed that somatic mutations are associated with tissue-wide tau pathology. These findings suggest that tau aggregation and somatic mutation accumulation occur independently, pointing to an upstream pathological event driving both processes. Moreover results indicate that tangles do not confer cell-autonomous genotoxicity to neurons and that non-tangle components drive somatic mutation in AD, with implications for tau-targeted therapy.
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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

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Program #/Poster #: PSTR064.12/D38

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

Support: NIH Grant 5R01AG0H2899

Title: Multiomic Insights into Inflammation-Driven Microglial Dysregulation in AD

Authors: *S. R. WROBLEWSKI[1], E. B. ENGLER-CHIURAZZI[2], K. ZWEZDARYK[3]; [1]Dept. of Microbiology & Immunol., Tulane Univ. Neurosci. Grad. Program, New Orleans, LA; [2]Neurosurg., Tulane Univ., New Orleans, LA; [3]Dept. of Microbiology & Immunol., Tulane Univ. Sch. of Med., New Orleans, LA

Abstract: Background Peripheral inflammation is linked to neurodegeneration. Using Xenium spatial transcriptomics (Fig. 1), we test the hypothesis that peripheral inflammation triggers transcriptional changes in microglia. Preliminary data show microglial transcriptional changes are detectable within days of the peripheral inflammatory event, and moreover, that these changes persist for months. How this impacts Aβ and tau pathology is unknown. Method 3xTg-AD mice were exposed to a peripheral (does not directly infect the CNS) viral infection inducing systemic inflammation. At 7 days- and 2-months post infection, the right and left hemispheres of the brain, respectively, were harvested for immunostaining and integrated analysis of Multiome sequencing (simultaneous single-cell RNA-seq and ATAC-seq) with spatial transcriptomics. Circulating cytokine levels in the blood were measured via ELISA. Results At the 7-day timepoint, spatial transcriptomics revealed 265 differentially expressed genes in microglia, with three unique microglial profiles present only in infected animals (Fig. 2). Cluster features, such as downregulated Plcxd2 and Bhlhe40, have been associated with intracellular Aβ and pTau, as well as dysregulated lipid processing, respectively. Further, algorithmic cell sorting revealed overlap of microglia with glutamatergic neurons and endothelial cells, suggesting increased microglial engulfment of these cell types. Preliminary scRNA-seq data at 2-months post-infection reveals a pro-inflammatory microglial phenotype (Fig. 3), as shown by increases in Ctss and Cd84. Genes critical for synaptic function and neuronal support (Snap25, Anks1b, Fam155a) were significantly downregulated, suggesting that microglia-neuron interactions are influenced by peripheral inflammation. These changes correlated with global increased expression of Apoe and Psen1&2. Together, these findings show that peripheral inflammatory events induce significant deviations in microglia homeostasis. Conclusion Our preliminary findings suggest that viral-induced peripheral inflammation alters microglia state and homeostasis in a 3xTg-AD model. Despite the clearance of systemic inflammation, the transcriptional profile of microglia remains different in infected vs mock-infected mice. The data suggest peripheral inflammatory stress events alter microglial transcriptional profiles and a "reset" to the original baseline profile is not immediate. The implications on neurodegeneration are being explored.

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Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

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Program #/Poster #: PSTR064.13/D39

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

Support: EENT Foundation of New Orleans to NGB

Title: Single-cell transcriptomics analysis of tauopathy and amyloidosis rat models for Alzheimer's Disease reveals synaptic dysfunctions in the hippocampus.

Authors: *B. GILES[1], S. BHATTACHARJEE[1], J. JI[1], M.-A. KAUTZMANN[1], S. DO CARMO[2], A. CUELLO[2], N. G. BAZAN[1];
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Abstract: Alzheimer's disease (AD) primarily affects the elderly population, leading to progressive cognitive decline and dementia. The AD hallmark pathology includes extracellular accumulation of amyloid-beta (Aβ) plaques, and intracellular neurofibrillary tangles (NFT) composed of hyperphosphorylated tau proteins. Understanding the mechanisms underlying AD is necessary for developing effective therapeutic strategies. We explored the genomic and epigenomic landscape of the hippocampus of novel rat models: Tauopathy and Amyloidosis using Single-Cell and Spatial Sequencing to detect the transcriptomic signatures. McGill-R955-hTau, McGill-R-Thy1-APP, and age-matched control rat hippocampi (n=3) were harvested, minced, and single nuclei was isolated by 10x Genomics Multiome Nuclei Isolation kit. 10x Chromium was used to obtain mRNA and chromatin accessibility profiling at single-cell resolution. The mRNA and ATAC libraries were sequenced using a NovaSeq 6000 system targeting at minimum 80,000 and 50,000 reads / cell for mRNA and ATAC respectively. McGill-R955-hTau and age-matched control coronal sections were transferred onto the fiducial frames of a Visium Spatial Gene Expression slide. Spatial barcoding and cDNA library was generated from the tissue mRNA, which was mapped to a 55 μm spatial resolution corresponding to 1-10 cells / spot. The mRNA library was sequenced using a NovaSeq 6000 platform to a minimum depth of 50,000 reads / cell. Single-Cell analysis revealed cell type specific differential gene expression highlighting synaptic dysfunctions within hippocampal neurons and synapse regulation changes in glial cells in both the Tauopathy and Amyloidosis models. Additionally, in Tau rats, ligand-receptor expression analysis revealed Netrin signaling dysfunction. Spatial sequencing analysis confirmed synaptic dysfunction in Tau rats, as well as revealing Neurotrophic signaling dysfunctions. In summary, this study successfully characterized cell types, identified synapse and cell-cell interaction dysfunctions through differential expression and cell type specific ligand-receptor expression, and uncovered genomic and epigenomic landscape of the hippocampus of a Tauopathy and in an Amyloidosis model of AD in rats.

Disclosures: B. Giles: None. **S. Bhattacharjee:** None. **J. Ji:** None. **M. Kautzmann:** None. **S. Do Carmo:** None. **A. Cuello:** None. **N.G. Bazan:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.14/D40

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

Support: 25-BR-02-04
25-BR-06-01

Title: Systematic proteogenomic analysis identifies causal plasma proteins and subtype-specific biomarkers for Alzheimer’s disease

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Abstract: Alzheimer’s disease (AD) is a multifactorial neurodegenerative disorder with high heritability and growing public health impact. Although recent studies have identified numerous blood-based biomarkers for early AD detection, the lack of causal evidence has limited their translational utility. We aimed to identify blood proteins with a potential causal role in AD using large-scale proteomic and genetic datasets. We conducted Mendelian randomization (MR) analyses using plasma pQTL summary statistics from 54,219 individuals and AD-related GWAS datasets, collectively covering over 1.1 million individuals. These GWAS covered diverse AD phenotypes, including clinically diagnosed AD, late-onset AD, tau levels, hippocampal volume, and cardiovascular comorbidities. To evaluate causality and directionality, we employed genetic epidemiology approaches and assessed genetic colocalization to identify shared causal signals. Among approximately 25,000 protein-trait pairs, 71 candidates showed consistent evidence of a directional relationship with AD-related phenotypes. Of these, 15 proteins were further supported by shared genetic architecture, highlighting their potential causal relevance. This study demonstrates a comprehensive proteogenomic approach to nominate and prioritize plasma proteins as putative biomarkers or therapeutic targets for AD.

Disclosures: H. Jung: None. **S. Lee:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

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

Support: NIH Grant K99/R00 AG068306
Alzheimer's Association Research Grant AARG-23-1074289

Title: Spatial Transcriptomic Profiling of Memory-Responsive Gene Expression Impacted by Tau Pathology

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Abstract: Hippocampal memory consolidation relies on spatiotemporally regulated, distinct 'waves' of transcriptional events. With the advent of spatial transcriptomic profiling techniques, we recently mapped the learning induced gene expression changes in spatially distinct subregions of the dorsal hippocampus following a spatial object recognition (SOR) task. This study, however, lacked the cell-type specific information on transcriptional signatures of the dorsal hippocampus. Therefore, we employed the state-of-the-art Xenium platform to delineate the spatial patterns of learning-induced gene expression across hippocampal subregions at a single-cell resolution. We trained adult C57BL/6J male mice in the SOR task, and spatial gene expression analysis was performed on hippocampal sections one hour after training in the SOR task. We identified distinct as well as overlapping signatures of learning-induced genes including key engram-specific markers such as Arc, Nr4a1, Egr1, and Egr4, which were upregulated across several hippocampal neuronal cell types. We also observed distinct signatures of learning-responsive genes in hippocampal non-neuronal cells. The hippocampus is particularly vulnerable to the neuropathological features of Alzheimer's disease and related dementia (ADRD). One of the pathological hallmarks of ADRD is the hyperphosphorylation of the microtubule-associated protein Tau (MAPT). The impact of tau pathology on the hippocampal transcriptome during memory consolidation remains largely elusive. We employed a well-established rodent model of ADRD that expresses the mutant form of the human MAPT (P301L) gene in excitatory neurons. This model displays several key pathological features of ADRD along with deficits in hippocampal long-term spatial memory. Here, P301L and control transgenic mice were trained in the SOR task, and Xenium spatial transcriptomic profiling was performed one hour after training. Notably, key learning-responsive genes in neuronal and non-neuronal glial cells were found to be dysregulated in the hippocampus of P301L transgenic mice when compared to controls. To understand its relevance in the context of human AD, these findings were subsequently compared to the published single-nuclei RNA-Seq dataset derived from postmortem hippocampal samples of ADRD patients. Together, our study provides unique molecular insights into how tau pathology disrupts the signature of learning-responsive gene expression across the hippocampal cell types, suggesting a potential mechanism underlying memory decline associated with ADRD.

Disclosures: B. Basu: None. **S. Bliese:** None. **S. Beyer:** None. **S. Chatterjee:** None.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

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

Support: NIA Grant R44AG079691

Title: Single-cell CUT&Tag + RNA reveals region-specific epigenetic dysfunction in Alzheimer's Disease

Authors: C. HARTL[1], ***P. LIN**[2];
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Abstract: Epigenetic alterations are a major emerging focus area in Alzheimer's Disease (AD), in particular late-onset AD for which environmental factors play a substantial role in risk, limiting the explanatory power of genetics alone. While hundreds of AD-linked epigenetic differences have been identified, including DNA methylation, histone post-translational modifications, and alterations in 3D genome structure, little is known about how these alterations give rise either to cellular dysfunction or regional susceptibility in AD. Furthermore, a major challenge in AD epigenetics is disentangling compositional differences (differences due entirely to cell loss) from biologically relevant within-cell-type abnormalities. To meaningfully probe cellular and regional epigenetic changes in AD, we generate the first single-cell, multi-region, RNA+histone modification (mHist) atlas. We profile four key histone marks, tagging element activity (H3K27ac, H3K4me1) and repression (H3K27me3, H3K9me3) across three brain regions from 5 AD donors and 5 phenotypically normal controls. We selected tissue samples from Hippocampus (early neuronal vulnerability), peri-ventricular white matter (astrogliosis), and cerebellum (resiliency) to reflect differential susceptibilities of cell types to AD pathology. Utilizing Droplet Paired-Tag on the 10X Chromium platform, we uniquely profile both transcriptomic profiles and mHist profiles in each cell. Using these data, we (i) define region-, cell-, and (region x cell)-specific regulatory modules through analysis of chromatin states, and highlight pan-cerebral and region-restricted regulatory elements; (ii) investigate how enhancer-promoter interaction strength differs between regions for the same cell type; (iii) highlight relationships between epigenetic and transcriptional mechanisms of glial cell activation, a key inflammatory process in AD pathology; and (iv) comprehensively examine epigenetic erosion - a broad distortion of epigenetic cell state and chromatin regulation in AD - comprehensively across brain regions and epigenetic marks.

Disclosures: C. Hartl: A. Employment/Salary (full or part-time): Epigenome Technologies. **P. Lin:** A. Employment/Salary (full or part-time): Epigenome Technologies.

Poster

PSTR064: Alzheimer's Disease: Genomics Approaches

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR064.17/D43

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

Support: R01 AG059778

Title: Hypothalamic neuronal GPCR expression is altered due to aging and 5XFAD transgene

Authors: *J. ALGOO[1], A. DUNN[2], V. MENON[1], K. O'CONNELL[2];
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Abstract: The hypothalamus is a complex brain region that plays a large biological role in health and disease. It coordinates brain and body responses to environmental stimuli, and leads to stereotyped physiology characteristic of internal states like fear, hunger, and sexual arousal. In the context of aging and Alzheimer's Disease (AD), the hypothalamus is understudied, but patient symptomatology, which in part includes metabolic and circadian rhythm abnormalities, nominate a potential causal role for the region. To elucidate how age and AD affect the transcriptome profile of individual cell types within this region, we performed snRNAseq on 80 mice across 10 strains from the AD-BXD panel, evenly split by age (6/14 mos), sex, and transgene status (5XFAD/WT). We produced a dataset of 1.18M nuclei post-QC, identified genes and clusters that change due to age, 5xFAD transgene, cognitive performance, and their interactions with each other and with sex. In our neuronal clusters, we identified many GPCR genes that had significant associations with each of our variables of interest as well as with sex. Our results in Figure 1 display the number of significant GPCR associations (summed over all neuronal clusters) for 3 prominently altered classes of GPCRs in our dataset: adhesion, purinergic, and glutamate/glycine metabotropic GPCRs. We find more significant metabotropic and adhesion associations compared to purinergic ones for two comparisons we performed, evaluating age in WT mice (old WT vs young WT) and 5XFAD transgene in young mice (young 5XFAD vs young WT). Our results nominate a role for altered ligand-receptor sensing in the hypothalamus due to age/AD, mediated by changes in the expression of GPCRs.

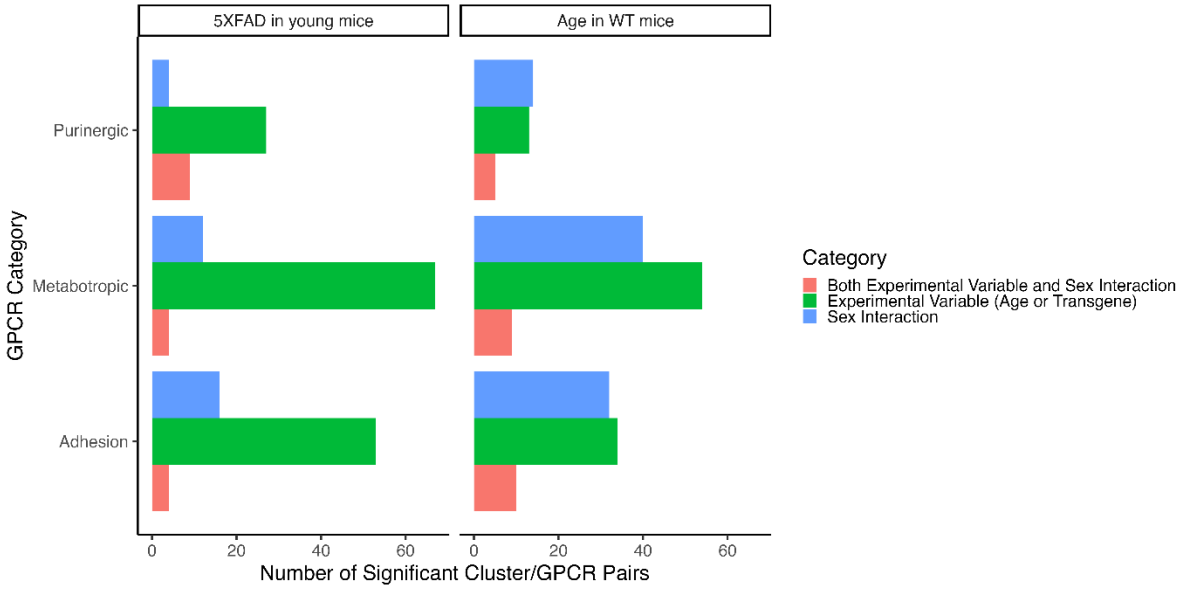


Figure 1. Number of significant differentially expressed genes across GPCR categories.

Disclosures: J. Algoo: None. **A. Dunn:** None. **V. Menon:** None. **K. O'Connell:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

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

Support: JPSP KAKENHI (21H02649)
JPSP KAKENHI (22H02786)
AMED BINDS (22ama121018)
AMED (23ak0101192h0001)

Title: High-purity brain capillary isolation from frozen mouse and human brains for quantitative proteomics

Authors: *S. ITO[1], S. OGATA[2], T. MASUDA[3], S. OHTSUKI[1];
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Abstract: Brain capillaries are crucial for understanding protein expression changes at the blood-brain barrier (BBB), but conventional isolation methods require pooling at least five mouse brains or large amounts of human brain tissue (>1 g) to obtain sufficient purity for protein analysis. In this study, we developed a method enabling the isolation of brain capillaries from a single frozen mouse brain or a small quantity of frozen human brain, making protein expression analysis feasible for limited or archived samples. Brain capillaries were isolated from frozen mouse and human brains using a bead homogenizer for tissue disruption, followed by purification with cell strainers and glass beads (developed method). For comparison, the standard method employed a Potter homogenizer for fresh mouse brain and nylon mesh purification. Using the developed method, we successfully isolated brain capillaries from a single frozen mouse brain, as confirmed by Western blot and proteomic analysis, which showed that BBB-expressed proteins were more highly enriched than in capillaries isolated from pooled mouse brains using the standard protocol. The developed method also enabled capillary isolation from as little as 0.3 g of frozen human brain tissue, with Western blot and proteomics demonstrating greater enrichment of BBB proteins compared to whole-brain lysate. Furthermore, analysis of cell type-specific markers indicated that the proportions of endothelial markers, and the reduction of neuronal markers, were similar between human and mouse samples, suggesting a significant enrichment of brain capillaries and minimal neuronal contamination in both. In summary, this newly developed protocol allows for efficient and high-purity isolation of brain capillaries from minimal frozen tissue samples, supporting robust protein expression analysis and providing a foundation for more detailed investigations of BBB physiology and pathology in both experimental animals and human brains. This method also provides a practical platform for translational studies in neurological diseases such as Alzheimer’s disease, by enabling proteomic analysis of archived animal models and human brain specimens.

Disclosures: S. Ito: None. **S. Ogata:** None. **T. Masuda:** None. **S. Ohtsuki:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.02/D45

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

Support: AMED 25wm0625001s0203

Title: Molecular Diversity of Abeta Seeds and Their Differential Impact on Parenchymal and Vascular Abeta Pathology in Alzheimer's Disease

Authors: *H. UCHIGAMI[1,2], E. YAMAUCHI[3], T. KAMAKURA[3], A. ARAKAWA[4], T. MATSUBARA[4], S. MURAYAMA[4], Y. SAITO[4], M. OHGOH[3], K. HORIE[3], T. IWATSUBO[2,1], T. HASHIMOTO[1,2];
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Abstract: β-amyloidosis is a key pathological feature of Alzheimer's disease (AD) brains, exhibiting considerable heterogeneity. The severity of cerebral amyloid angiopathy (CAA) varies significantly among AD brains. We hypothesized that this pathological diversity may be attributed to differences in the properties amyloid β (Aβ) seeds. This study aimed to elucidate how distinct properties of Aβ seeds influence the pathological diversity. We analyzed 32 tissue samples from 16 AD autopsy cases exhibiting varying degrees of CAA severity. We extracted high-molecular-weight Aβ oligomers (HMW Aβ seeds) with Aβ pathology-inducing properties from brain parenchyma and meninges/vessels. To evaluate seeding capacity, we injected these Aβ seeds were into AD model mice, and evaluated Aβ deposition patterns after 4 months. Quantitative analyses revealed that HMW Aβ seeds were more abundant in CAA-rich cases than in CAA-minimal cases and in meningeal and vascular tissues than in brain parenchyma. These results suggest that HMW Aβ seeds are abundant in the meninges and vessels, particularly in CAA. Seed inoculation experiments revealed distinct seeding profiles. Aβ seeds from CAA-rich cases exhibited a greater tendency to induce vascular Aβ deposition (CAA), whereas seeds from CAA-minimal cases primarily induced parenchymal Aβ deposition. Additionally, a significant correlation was found between the plaque-to-CAA ratio in human AD brains and the vascular or parenchymal seeding properties of the extracted Aβ seeds. To investigate the factors contributing to these distinct seeding profiles, we performed co-immunoprecipitation mass-spectrometry (Co-IP-MS), which revealed that certain proteins were enriched in HMW Aβ seeds with CAA-inducing properties. Taken together, these results suggest that different pathology-inducing properties of HMW Aβ seeds may reflect the diversity of Aβ pathology in AD brains. These properties may reflect differences in their associated protein interactions, which requires validation through *in vivo* studies.

Disclosures: H. Uchigami: None. **E. Yamauchi:** None. **T. Kamakura:** None. **A. Arakawa:** None. **T. Matsubara:** None. **S. Murayama:** None. **Y. Saito:** None. **M. Ohgoh:** None. **K. Horie:** None. **T. Iwatsubo:** None. **T. Hashimoto:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.03/D46

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

Support: Akiyama Life Science Foundation

Title: Preserved function of choroid plexus in the cognitive normal subjects with Alzheimer pathology in postmortem human study

Authors: *K. KIHARA[1], M. NAKANO[3], E. KOBAYASHI[2], K. NAGAISHI[2];
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Abstract: The neurodegeneration caused by Alzheimer’s disease (AD) is believed to be triggered by an accumulation of amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs). Although several postmortem studies have reported cognitive normal subjects with AD pathology, little is known about the mechanisms underlying these discrepancies. In this study, we aim to determine brain barrier functions in cognitively normal subjects with AD pathology. We analyzed postmortem brain samples from nine individuals aged 87 to 103, obtained through the body donation program at Sapporo Medical University. AD pathology was diagnosed by the NIA-AA criteria, which evaluates Thal Aβ phase, Braak NFT stage and CERAD neuritic plaque score. Cognitive function was evaluated using Clinical Dementia Rating (CDR). According to these criteria, the brains were classified into two groups: (1) AD pathology with No dementia; AD-N (n= 4), and (2) AD pathology with Dementia; AD-D (n= 5). The protocols were approved by the Sapporo Medical University Ethics Committee (26-2-51). The Aβ- and PHF-tau-positive areas in the entorhinal cortex (EC) in AD-N were as high as AD-D. However, the number of NeuN-positive cells, the synaptophysin-positive area, the number of GFAP-positive processes and the GLT-1 positive area in the EC were significantly higher in the AD-N than AD-D. To evaluate the function of brain barriers, we focused on cell adhesion of the choroid plexus (CP), that form blood-cerebrospinal fluid barrier (BCSFB). According to the real-time PCR and immunohistochemistry of the CP, gene and protein expression of ZO-1 and E-cadherin were significantly higher in the AD-N than AD-D. However, there was no difference in the expression of those adhesion molecules in hippocampus, that contain vessels forming the blood brain barriers. We found in AD-N group, the maintenance of choroid epithelial cells leads to the preserved neuronal number, synaptic density and astroglial function despite of their strong AD pathology. It is suggested that the mechanism of blocking the entry of abnormal substances into

CNS influences cognitive function of people with AD pathology. These histological analysis on AD-N suggested that the integrity of BCSFB affected pathophysiologic progression of AD.

Disclosures: K. Kihara: None. **M. Nakano:** None. **E. Kobayashi:** None. **K. Nagaishi:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.04/D47

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

Title: Explore the impact of vascular pathology on neuronal activity in the CADASIL mouse model.

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Abstract: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a cerebral small vessel disease caused by mutations in the *NOTCH3* gene. The NOTCH3 gene is expressed in vascular smooth muscle cells (VSMCs), which succumb in CADASIL patients leading to arteriopathy and subcortical ischemic infarcts. Many CADASIL patients however also suffer from cognitive decline, indicating that neuronal functions are disturbed, but less is known about the cellular basis for the cognitive aspects of CADASIL.
In this study, we investigated the mechanisms of neuronal dysfunction using human postmortem CADASIL brain sections and a humanized CADASIL mouse model (R182C-TgN3). We analyzed neural fiber densities through immunohistochemistry. Mitochondrial function in the hippocampus and brain vasculature of R182C-TgN3 mice, as well as in primary human VSMCs, was assessed using western blot and Seahorse assays. The expression of inflammation-related genes was analyzed using qPCR. Neuronal activity in R182C-TgN3 mice was evaluated by measuring gamma oscillations with electrophysiology. Additionally, we visualized vascular and microglial architecture using the iDISCO+ method on the whole mouse brain.
We observed significant decrease and alterations in neuronal fiber length and aberrant morphology in the cortex and hippocampus of both human CADASIL brains and the R182C-TgN3 mice by immunostaining with the pan-neuron markers ubiquitin C-terminal hydrolyse L1 (UCHL1) and neurofilament heavy chain (NFH). The R182C-TgN3 mice also exhibited impaired hippocampal gamma oscillation pattern, indicating disturbed neuronal firing in the CADASIL mice. Additionally, there were lower levels of mitochondrial respiratory complexes in the hippocampus and isolated brain vessels. Consistent with these findings, primary VSMCs harboring the R133C CADASIL mutation also exhibited reduced levels of respiratory complexes, oxygen consumption rates, and ATP levels, alongside increased pro-inflammatory gene expression (CCL2, CCL5, ICAM). This suggests a broader impact on cellular energy

metabolism features that are indicative of a neuroinflammatory process. iDISCO+ analysis also revealed reduced vessel wall coverage and increased vessel-associated microglia.
This study highlights potential vascular and immune system involvement in the observed neurological deficits in CADASIL. We provide evidence for extensive neuronal dysfunction in CADASIL, which may explain the cognitive decline observed in later stages of the CADASIL disease process.

Disclosures: W. Shao: None. **H. Karlström:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

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Program #/Poster #: PSTR065.05/D48

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

Support: NIH R01 AG074999
NIH R01 AG81774

Title: Hippocampal hypoperfusion in vivo and impaired microvascular responses in vitro in CVN mouse model of Alzheimer’s Disease

Authors: S. DEGAN[1], Q. LI[2], ***D. A. TURNER**[1,3];
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Abstract: Insufficient metabolic supply can enhance the progression of Alzheimer's disease [AD], if reduced neurovascular coupling and cerebral blood flow [CBF] responses cannot meet energy demands of activated neurons. However, correlation is needed between dynamic CBF responses and underlying microvascular function in hippocampus. Transgenic CVN mice [APPSwDI +/+ mNos2−/−] exhibit critical pathological features resembling human AD, such as peri-vascular amyloid deposition, phosphorylated tau, reduced neurovascular coupling, and hippocampal neurodegeneration, rendering it an appropriate model for investigating AD pathophysiology. We analyzed synaptic stimulation of the hippocampus *in vivo*, measuring CBF responses using laser speckle imaging, comparing middle-aged (30 - 40 weeks old) CVN to control C57Bl/6 [WT] mice. Hippocampal CBF responses were quantitated using regions of interest in the exposed hippocampus during synaptic stimulation with a train of 20 Hz pulses (0.25 ms pulse width) for 5s, between 0.25 and 1.5 mA. We further assessed hippocampal capillary diameter in response to pharmacological stimulation in *in vitro* acute brain slices (300 μm thickness) of similar ages, using norepinephrine to first vasoconstrict capillaries, then glutamate to secondarily dilate microvessels. We found that, when compared with age-matched WT mice, middle-aged CVN mice exhibited a significant reduction in focal hippocampal CBF at all intensities tested, indicative of a deficiency in neurovascular coupling and resulting in hypoperfusion. Additionally, we measured changes in the diameters of visualized capillaries in

acute hippocampal slices under IR-DIC microscopy with digital imaging. In these capillaries (in stratum radiatum of slices from middle-aged CVN and WT mice) bath application of the potent vasodilator glutamate (0.5mM) completely restored pre-constricted hippocampal capillaries to prior diameters in middle-aged WT mice, but failed to induce vasodilation in age-matched CVN mice. These results indicate severe impairment in both macrovascular (CBF) and microvascular (capillary diameter) vasodilatory responses to neuronal stimulation and glutamate in middle-aged CVN mice. Our present findings strongly suggest that compromised microvascular function may underlie CBF hypoperfusion observed in CVN mice to synaptic stimulation and spreading depolarization. Therefore, early restoration of neurovascular coupling in the timeline of AD may improve AD outcome. We are pursuing this goal with both whole brain transcranial AC stimulation as well as improving pericyte K+ channel function.

Disclosures: S. Degan: None. **Q. Li:** None. **D.A. Turner:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

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Support: 1R01AG081258-01A1
1RF1NS132291-01
1R01AG081874-01
22TPA970253
24POST1196128
25POST1373581

Title: Modulation of the nitric oxide pathway by protein arginine methyltransferase in a mouse model of sickle cell disease

Authors: *J. ZACCARELLI MAGALHÃES[1], M. UDO[2], D. J. SMITH[3], L. H. MATUGUMA[3], J. LANGMAN[4], G. NAGAMINE GOMES[4], P. RODRIGUEZ[4], C. T. CITADIN[3], H. LIN[3];
[1]Univ. of Texas, Houston, TX; [2]Neurol., UT Hlth. at Houston, Houston, TX; [3]Neurol., [4]UTHealth Houston, Houston, TX

Abstract: Background: Sickle cell disease (SCD) is a hereditary blood disorder caused by a point mutation of the β-globin gene, affecting approximately 100,000 individuals in the United States and over 306,000 worldwide, predominantly among individuals of African heritage. In this disease, hemoglobin polymerizes, causing the cells to assume a rigid sickle-shape. The abnormal hemoglobin does not properly carry enough oxygen through the body leading to serious cardiovascular alterations, including the development of vascular dementia - characterized by cognitive and memory impairment and reduced cerebral perfusion. Although

SCD affects both men and women, sex-specific differences remain unclear due to a lack of research. Thus, the aim of this study is to investigate the mechanism(s) underlying vascular dementia in both female (F) and male (M) Townes mice (model of SCD); focusing on the role of arginine methyltransferase proteins (PRMTs), an important enzyme family involved in neurovascular regulation and promising therapeutic target for SCD. **Hypothesis**: Dysregulation of PRMTs in SCD leads to impaired nitric oxide signaling, neurovascular coupling deficits and loss of memory and cognitive function in both sex Townes mice. **Results**: Our results suggest that 1) both sex Townes mice exhibited elevated levels of Type I PRMTs (F: [PRMT1: $p=0.018$; PRMT4: $p=0.019$; PRMT6: $p=0.011$; PRMT8: $p=0.0029$]; and M: [PRMT4: $p=0.020$]), asymmetric dimethylarginine (ADMA) (F: $p=0.05$; M: $p=0.004$), and dimethylarginine dimethylaminohydrolase 1 and 2 (DDAH1 and 2) (F DDAH1: $p=0.005$; DDAH2: $p=0.029$), along with increased endothelial nitric oxide synthase (eNOS) (F: $p=0.035$) and decreased inducible nitric oxide synthase (iNOS) (F: $p= 0.0135$; M: $p<0.0001$). 2) Two-photon laser scanning microscopy combined with whisker stimulation suggest neurovascular coupling dysfunction in Townes mice, as indicated by reduced microvessel diameter following stimulation (F: $p=0.009$; M: $p=0.019$). 3) Both sexes of Townes mice presented reduced mobility (F: $p<0.01$; M: $p<0.0001$), increased anxiety-like behavior (F: $p<0.01$; M: $p<0.0001$), and impaired short (F: $p<0.01$; M: $p<0.05$), and long-term memory (F: $p<0.0001$; M: $p<0.0001$). **Conclusions**: Townes mice exhibit molecular, vascular, and behavioral abnormalities, with females more affected than males. Our findings suggest dysregulation of the nitric oxide signaling by Type I PRMTs, leading to vascular deficits and decreased memory and cognitive function. These results highlight the potential role of PRMTs and the nitric oxide pathway in neurovascular and behavioral phenotypes associated with SCD.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.07/D50

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

Support: NIH Grant AG052350
NIH Grant NS034467
Cure Alzheimer's Fund

Title: APOE4 and Tau P301S act synergistically to increase the severity of vascular pathology and neurodegeneration

Authors: *C. TORRES SEPULVEDA[1], M. ZHANG[2], A. P. SAGARE[3], R. RUST[2], A. CHAKHOYAN[4], K. KISLER[5], V. CLEMENTEL[1], G. SPILLARD[3], D. M. HOLTZMAN[6], B.

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Abstract: Early blood-brain barrier (BBB) breakdown occurs in Alzheimer's disease (AD) and is thought to contribute to cognitive impairment. The E4 allele of the *APOE* gene, *APOE4*, is the major genetic risk factor for AD, compared to *APOE3* that carries lower risk. *APOE4* exerts substantial cerebrovascular toxicity including BBB breakdown both in humans and in *APOE* transgenic models, which we previously showed precedes cognitive impairment and synaptic deficits. Here, we studied BBB and neurodegenerative changes in 9.5-month-old *APOE3* and *APOE4* knock-in floxed (F) mice, i.e., *E3F* and *E4F,* alone and crossed with a mouse model of tauopathy (*P301S*). Immunostaining for blood-derived fibrinogen revealed increasing BBB leakage in *E4F* compared to *E3F* mice, which was further exacerbated by tauopathy in *E3F; P301S* and *E4F; P301S* crosses, with the most severe BBB leakage observed in the *E4F; P301S* mice. DCE-MRI at 9.5-months also confirmed changes in BBB permeability. Neurodegenerative changes at 9.5-months were also present and followed a similar isoform-dependent pattern as BBB changes, with reduced hippocampal neuronal density in *E4F* compared to *E3F* mice, and further degeneration in mice with tauopathy, with *E4F; P301S* mice exhibiting the greatest loss of neuronal density. *In vivo* T2w-MRI at 9.5-months also revealed regional brain atrophy consistent with neurodegeneration. Proteomic and phosphoproteomic analysis of PSD fractions confirmed substantial synaptic protein loss and dysregulation of synaptic signaling networks. The synaptic phenotypes followed a similar pattern with a synergic increase in the severity with *E4F; P301S* mice most affected. Together this data suggests that BBB breakdown and neurodegenerative changes are more severe in *APOE4* compared to *APOE3* mice, and *APOE4* expression combined with tau pathology synergistically increase the severity of BBB and neurodegenerative pathology.

Disclosures: C. Torres Sepulveda: None. **M. Zhang:** None. **A.P. Sagare:** None. **R. Rust:** None. **A. Chakhoyan:** None. **K. Kisler:** None. **V. Clementel:** None. **G. Spillard:** None. **D.M. Holtzman:** None. **B. Zlokovic:** None. **H.N. Yassine:** None. **M.P. Coba:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.08/D51

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

Support: 1R01AG081258-01A1
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1R01AG081874-01
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Title: Pericyte Protein Arginine Methyltransferase 5 is Reduced After Oxygen Glucose Deprivation

Authors: *D. J. SMITH, C. T. CITADIN, J. ZACCARELLI MAGALHÃES, L. H. MATUGUMA, J. LANGMAN, H. LIN, M. UDO;
Neurol., UTHealth Houston, Houston, TX

Abstract: Background: Protein arginine methyltransferases are key epigenetic regulators whose role in the cerebrovascular system remains poorly understood. Pericytes, prominent regulators of the blood-brain barrier (BBB) function and cerebral blood flow, have been implicated in Alzheimer's disease (AD) pathology. Previous work from our lab has shown that inhibition of these PRMTs can increase cerebral blood flow. Given that pericyte dysfunction contributes to neurovascular deficits in Alzheimer's disease (AD) and ischemic injury, we examined PRMT isoforms in human brain pericytes and identified PRMT5 as the most highly expressed. We then examined PRMT5 regulation *in vitro* under ischemic stress and in the 3xTg-AD mouse model. **Methods:** Cultured human brain vascular pericytes and endothelial cells were plated separately and grown, pericytes were assessed for PRMT mRNA levels by RT-qPCR across all PRMT isoforms (1-9). To model ischemic stress, pericytes were subjected to oxygen-glucose deprivation (OGD) for 3 hours. Protein expression of PRMT5 and its primary end-product symmetric dimethyl arginine (SDMA, a measurement of activity) were quantified by capillary electrophoresis (ProteinSimple™ Jess). Cortex and hippocampus were harvested from young (3-4 months) and aged (9-12 months) 3xTg-AD female and male mice, as well as aged-matched C57BL/6J controls. PRMT5 protein levels were measured and compared by one-way ANOVA, unpaired t-test, or Tukey's post hoc test. **Results:** Among PRMT isoforms 1-9, PRMT5 mRNA expression (10.81±1.03 AU) was significantly higher than any other PRMT mRNA expression (1.02±0.08 AU; $p<0.01$). PRMT5 relative protein expression was significantly higher in pericytes (0.224±0.009 AU) v. endothelial cells (0.104±0.005), to suggest pericyte specificity ($p<0.05$). Further analyses suggest that under OGD conditions, PRMT5 expression reduced significantly (-0.14±0.00 AU; $p<0.05$), as does its primary end-product, SDMA (-0.01±0.00 AU; $p<0.05$) a measurement of PRMT5 activity. PRMT5 was also decreased in aged female vs. young female 3xTg-AD in both cerebral cortex and hippocampus brain regions (both $p<0.05$). **Conclusion:** PRMT5 is the predominant PRMT in human brain vascular pericytes and is downregulated by ischemic stress in an age and sex dependent manner in the 3xTg-AD model. These findings implicate that PRMT5 dysregulation play a role in Alzheimer's disease pathology and cerebrovascular deficits, highlighting PRMT5 modulation as a potential therapeutic target.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.09/D52

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

Support: AHA- 25PRE1365157
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NIH- AG057842
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TRIBA/Physiology Faculty Startup Fund from Augusta University.

Title: Sglt2 inhibition restores cerebral blood flow autoregulation and cognition via calcium homeostasis in alzheimer's disease

Authors: *A. GREGORY[1], S. WANG[2], J. XU[1], G. MORGAN[1], C. TANG[1], C. CANTWELL[1], D. BUNN[1], R. J. ROMAN[2], F. FAN[1];
[1]Physiol., Med. Col. of Georgia- Augusta Univ., Augusta, GA; [2]Pharmacol., Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: SGLT2 Inhibition Restores Cerebral Blood Flow Autoregulation and Cognition via Calcium Homeostasis in Alzheimer's Disease Andrew Gregory[1], Shaoxun Wang[2], Jing Xu[1], Gilbert Charles Morgan[1], Chengyun Tang[1], Cameron Cantwell[1], David Bunn[1], Richard J. Roman[2], Fan Fan[1,2] [1] Physiology, Medical College of Georgia, Augusta University, Augusta, GA. [2] Pharmacology, University of Mississippi Medical Center, Jackson, MS AD/ADRD is a major global health challenge and a significant burden, with no effective cure to date. Impaired cerebral blood flow (CBF) autoregulation and brain hypoperfusion are among the earliest pathological features of AD/ADRD. We previously reported that inhibition of SGLT2 significantly improved CBF autoregulation, cerebral perfusion, and cognitive performance in AD (Tg-F344AD) and diabetes-related ADRD (T2DN) rats. However, unlike in T2DN rats, SGLT2 inhibition did not affect plasma glucose levels in AD rats, suggesting that the underlying mechanisms may differ between AD and diabetes-related ADRD. To further investigate the vascular mechanisms specific to AD, we conducted the present study. We confirmed that SGLT2 is highly expressed in cerebral vascular smooth muscle cells (VSMCs), as well as in brain microvascular pericytes and endothelial cells, but not in astrocytes. Bulk RNA sequencing of primary cerebral VSMCs isolated from Tg-F344AD rats revealed that SGLT2 inhibition reversed dysregulated molecular pathways involved in VSMC contractility and vascular tone. These pathways include key regulators of actin cytoskeleton dynamics, oxidative phosphorylation, tight junction integrity, focal adhesion, AGE-RAGE signaling, and ECM-receptor interactions, all critical for maintaining vascular structure and function. Using Fluo-4 AM immunocytochemistry, we observed elevated intracellular calcium in primary AD VSMCs compared to controls. SGLT2 inhibition significantly reduced intracellular calcium levels in AD VSMCs. We also found that expression of the NHE1 was elevated in AD VSMCs and was reduced following SGLT2 inhibition. These findings suggest that the beneficial effects of SGLT2 inhibition on CBF autoregulation and cognitive function in AD may be mediated by modulation of VSMC calcium dynamics, potentially via NHE1 signaling. Altogether, our results support further clinical exploration of the vascular and neuroprotective potential of SGLT2 inhibitors in both diabetic and non-diabetic forms of AD, acting through mechanisms beyond glycemic regulation.

Disclosures: A. Gregory: None. **S. Wang:** None. **J. Xu:** None. **G. Morgan:** None. **C. Tang:** None. **C. Cantwell:** None. **D. Bunn:** None. **R.J. Roman:** None. **F. Fan:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: PSTR065.10/D53

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

Support: R01AG078859

Title: Impact of Directly Reprogramed Age- and Disease-Associated Microglia on the Blood-Brain Barrier

Authors: *R. GRANT[1], T. LAROCCA[2];
[1]Colorado State Univ., Fort collins, CO; [2]Colorado State Univ., Fort Collins, CO

Abstract: Brain aging is a complex biological process characterized by pathological events that intersect with and increase susceptibility to most neurodegenerative diseases, including Alzheimer’s disease (AD). Among these adverse events, blood-brain barrier (BBB) dysfunction is a key consequence of healthy brain aging that is also increasingly recognized as an early and significant event in AD. The BBB is vital for maintaining central nervous system (CNS) homeostasis, and pathological age-associated BBB changes correlate with cognitive decline and increased susceptibility to disease. Although the BBB is composed primarily of brain microvascular endothelial cells on the systemic surface with pericytes and astrocytes at the CNS interface, microglia (the brain’s resident immune cells) have been reported to modulate the BBB. However, age- and disease-associated changes in microglial-BBB crosstalk are incompletely understood. In this study, to better understand the impact microglia have on the BBB with aging and AD, we are directly reprogramming young, old, and AD peripheral blood mononuclear cells into monocyte-derived microglia-like (MDMi) cells. We are co-culturing these MDMis in a novel *in vitro* BBB model consisting of a human cerebral microvascular endothelial cell line (hCMEC), and evaluating the effects of MDMi-secreted cytokines and chemokines on barrier permeability and function. We are characterizing basic age/AD- and function-related aspects of directly reprogramed microglia and our *in vitro* BBB model via protein expression measurements. Also, to better understand how age- and AD-associated microglia affect the integrity of the BBB, our ongoing experiments will assess BBB permeability, central and systemic secretory factors, and leverage transcriptomics (RNA-seq) to broadly characterize underlying mechanisms.

Disclosures: R. Grant: None. **T. LaRocca:** None.

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PSTR065: Vasculature, BBB, and Alzheimer's Disease

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Program #/Poster #: PSTR065.11/D54

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

Support: NIH R41 AG084421
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Title: Generation of brain microvascular endothelial cells (BMECs) from induced pluripotent stem cells (iPSCs) for modeling brain vascular dysfunction in Alzheimer's disease

Authors: *C. HOWARD[1], N. BUTELET[1], A. DUONG[1], W. W. POON[1,2];
[1]NeuCyte, Inc., Mountain View, CA; [2]Inst. for Memory Impairments and Neurolog. Disorders, UC Irvine, Irvine, CA

Abstract: The blood brain barrier (BBB) is a critical structure separating blood flow from brain tissue. Brain microvascular endothelial cells (BMECs) make up a major component of the BBB by lining the blood capillaries in the brain and expressing tight junction proteins which serve as a physical barrier to restrict the passage of most molecules into the brain parenchyma. Breakdown of the BBB is implicated in numerous central nervous system (CNS) diseases including the early clinical stages of Alzheimer's disease (AD) before the onset of dementia. The E4 variant of apolipoprotein E (APOE4), a major genetic risk factor for AD, has been shown to lead to accelerated cerebrovascular dysfunction and BBB breakdown. This relationship between the BBB and clinical features of AD highlights the need to understand the BBB's role in AD pathophysiology and its potential as a therapeutic target. To address this need, we have developed methods for generating cryopreserved BMECs from various iPSC patient donors and gene-edited samples including from APOE4 (ε4/ε4) patients. Upon thaw, BMECs were highly pure, exhibited characteristic endothelial cell morphology, expressed tight junction proteins, and displayed typical vascular responses to inflammatory and VEGF-mediated stimuli. These BMECs spontaneously form vascular networks in 3D cultures and can be successfully co-cultured with other neural cells leading to more physiologically relevant *in vitro* systems. BMECs from disease-relevant patient backgrounds can be assessed through various high-throughput *in vitro* assays including angiogenesis assays, transepithelial electrical resistance (TEER) readouts, tube formation assays, and scratch-wound cell migration assays. These approaches have the potential for serving as high-throughput screening assays for BBB dysfunction in AD research and therapeutic development.

Disclosures: C. Howard: A. Employment/Salary (full or part-time): NeuCyte, Inc. **N. Butelet:** A. Employment/Salary (full or part-time): NeuCyte, Inc. **A. Duong:** A. Employment/Salary (full or part-time): NeuCyte, Inc. **W.W. Poon:** A. Employment/Salary (full or part-time): NeuCyte, Inc., UC Irvine.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

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Program #/Poster #: PSTR065.12/D55

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

Support: TCMMP 112-01-01

Title: Differential CSF biomarkers are associated with distinct microRNA expression profiles in idiopathic normal pressure hydrocephalus

Authors: *H.-Y. HUANG[1,2], S.-T. TSAI[3];
[1]Dept. of Med. Res., Hualien, Taiwan; [2]Dept. of Med. Res., [3]Depart of Neurosurg., Hualien Tzu Chi Hosp., Hualien, Taiwan

Abstract: Background: Idiopathic normal pressure hydrocephalus (iNPH) shows clinical and radiological features, but its underlying pathophysiological mechanisms remain incompletely understood. Given the overlap in biomarkers between iNPH and neurodegenerative diseases such as Alzheimer's disease, it is critical to identify molecular signatures unique to iNPH. Objective: This study aimed to investigate the expression patterns of microRNA (miRNA) in iNPH patients, stratified by two cerebrospinal fluid (CSF) protein biomarkers: amyloid beta 42 (Aβ42) and platelet-derived growth factor receptor beta (PDGFRβ). These biomarkers reflect pathological changes in amyloid metabolism and cerebrovascular function, respectively. Methods: Patients were classified into subgroups based on CSF Aβ42 and PDGFRβ levels. These biomarkers served as the analytical basis for investigating associated changes in miRNA expression. High-throughput sequencing was used to identify differentially expressed miRNAs, and RT-qPCR was used to validate the selected candidate miRNAs. Functional prediction was performed using pathway enrichment analysis. Results: Patients with lower Aβ42 and higher PDGFRβ levels tended to have severe ventriculomegaly, white matter changes, and impaired BBB function. Stratification by Aβ42 and PDGFRβ revealed subgroups with distinct biomarker profiles. These groups showed differential expression of specific miRNAs. Functional enrichment analysis suggested that these different miRNAs associated with biomarkers may be involved in regulating cellular senescence, apoptosis, extracellular matrix remodeling, and vascular signaling pathways. Conclusion: This study highlights the critical role of CSF protein biomarkers as a stratification framework for molecular subgroup analysis of iNPH. Identification of differentially expressed miRNAs can provide insights into the underlying regulatory mechanisms associated with disease-related molecular and structural brain changes. These findings provide an investigative strategy for developing CSF biomarker-based early diagnosis, risk prediction, and therapeutic targets in iNPH.

Disclosures: H. Huang: None. **S. Tsai:** None.

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PSTR065: Vasculature, BBB, and Alzheimer's Disease

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

Support: NIH–R01NS113912

Title: Amyloid-β Oligomers exposed astrocytes induce iron release from endothelial cells at the blood-brain barrier via extracellular vesicles

Authors: *K. PALSA[1], J. R. CONNOR[2];
[2]Neurosurg., [1]Penn State Col. of Med., Hershey, PA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative condition, clinically characterized by memory impairment and cognitive decline, and pathologically defined by the presence of amyloid-β (Aβ) plaques and neurofibrillary tau tangles. Alongside these hallmark pathologies, excessive brain iron accumulation is often observed in AD patients, occurring early in the disease. However, the release of iron from blood-brain barrier endothelial cells (BBBECs) through ferroportin 1 (FPN1) is inhibited by the pro-inflammatory cytokines TNF-α and IL-6, as well as hepcidin, which are released by astrocytes. The mechanism of iron accumulation in the brain remains unclear in the absence of FPN1. Our recent study demonstrated that endothelial cells also release iron to the brain via extracellular vesicles (EVs). In the current study, we utilized induced pluripotent stem cell (iPSC)-derived BBBECs and astrocytes in co-culture experiments to investigate EV-mediated iron release from endothelial cells in AD. The results indicate that exposure to Aβ oligomers (AβO) activates astrocytes, which subsequently release pro-inflammatory cytokines and hepcidin. Additionally, in co-culture experiments, AβO-activated astrocytes degrade FPN1 and enhance the expression of CD63, which is essential for EV synthesis. Furthermore, treatment on the basolateral side with pro-inflammatory cytokines increased the release of Fe57 in EVs and ferritin heavy chain 1 (FTH1) to the basolateral side compared to the control group. In co-culture experiments, AβO-activated astrocytes also increased the uptake of EV Fe57 relative to the control. These findings suggest that CD63+EVs released from blood-brain barrier endothelial cells constitute a major iron delivery pathway to the brain during AD.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: PSTR065.14/D57

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

Title: New frontiers in AD and blood-brain barrier (BBB) modeling: the APOE3 or APOE4 x Arte10 transgenic model and humanized BBB platforms for biological drug discovery.

Authors: S. R. STERLACE, E. GIUNTI, *J. R. RICHARDSON;
Taconic Biosci., Rensselaer, NY

Abstract: Preclinical mouse models for neurodegeneration are critical in drug discovery for Alzheimer's Disease (AD). Although limits exist in the predictive validity of these models, they allow insights into etiology and effects of genetic mutations and therapeutic modalities. With approval of human monoclonal antibodies, the first disease-modifying therapeutics for AD, there is demand for macromolecule therapeutic modalities. However, the blood brain barrier limits monoclonal antibodies' access to the brain. To address this, we created four new models: two for AD context in the presence and absence of a genetic risk variant (apolipoprotein E) and two for access to the brain via expression of targets for human mAbs receptor-mediated transcytosis (RMT). Vector constructs for humanization of solute carrier family 3 member 2 (SLC3A2; CD98 heavy chain) and transferrin receptor (TFRC) were transfected into mouse ES cells. BALB/c blastocysts were isolated, transferred to NMRI females, and resulting chimeric mice were bred to C57BL/6 females. Genotyping verified the constitutive humanized allele after deletion of the wildtype allele. RNA from tissues were analyzed by qPCR. Splenocytes were collected and analyzed by flow cytometry. To develop an AD context, two models (Arte10 and hAPOE4; on C57BL/6NTac background) were crossbred for investigation of human amyloid beta and APOE interaction. Whole genome sequencing identified the Arte10 transgene integration sites of the co-injected fragments (APPswe and PSEN1) within a segment of the mouse Thy1 gene. Arte10 mice were mated with human APOE4 and APOE3 knock-ins. Resulting mice had tissues collected for RNA analysis by qPCR.HOM humanized Tfrc and CD98hc mice both show human mRNA tissue-specific expression of their respective gene, 50% at WT murine levels and the other 50% at 0.5 to 2-fold difference. hTfrc splenocytes show internalization of fluorescent-conjugated human transferrin confirming the ability to endocytosis, a result not seen in WT mice. hCD98hc WT mice show expression of murine CD98hc in splenocytes whereas HOM only showed human expression. Arte10 HOM:HOM mice for APOE3 or APOE4 isoform, show gene and tissue-specific mRNA expression correlated to the expected genotype. The integration of human APOE variants to evaluate interactions on pathogenic amyloid beta production results in viable HOM offspring. These models can be bred to integrate multiple RMT options in the context of APOE and amyloid beta interactions in AD pathophysiology for therapeutic investigation of human mAbs in a model for evaluating amyloid-beta and/or apolipoprotein-directed effects.

Disclosures: S.R. Sterlace: A. Employment/Salary (full or part-time): Taconic Biosciences. **E. Giunti:** A. Employment/Salary (full or part-time): Taconic Biosciences. **J.R. Richardson:** A. Employment/Salary (full or part-time): Taconic Biosciences.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

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NIH- P20GM104357
TRIBA/Physiology Faculty Startup Fund from Augusta University.

Title: Inhibition of soluble epoxide hydrolase enhances the cerebral vascular function and attenuates cognitive impairment in diabetes-associated ADRD

Authors: G. MORGAN[1], C. TANG[1], S. WANG[2], J. XU[1], A. GREGORY[1], C. CANTWELL[1], D. BUNN[1], R. J. ROMAN[2], *F. FAN[1];
[1]Physiol., Med. Col. of Georgia at Augusta Univ., Augusta, GA; [2]Pharmacol., Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Inhibition of Soluble Epoxide Hydrolase Enhances the Cerebral Vascular Function and Attenuates Cognitive Impairment in Diabetes-Associated ADRD Gilbert Charles Morgan[1], Chengyun Tang[1], Shaoxun Wang[2], Jing Xu[1], Andrew Gregory[1], Cameron Cantwell[1], David Bunn[1], Richard J. Roman[2], Fan Fan[1,2] [1] Physiology, Medical College of Georgia, Augusta University, Augusta, GA. [2] Pharmacology, University of Mississippi Medical Center, Jackson, MS Recent human genetic evidence indicated that soluble epoxide hydrolase (sEH) is linked to AD/ADRD. Preclinical studies show that sEH inhibition improves cognitive function in AD models, likely via anti-inflammatory and neuroprotective mechanisms. We were the first to demonstrate that chronic sEH inhibition with TPPU reduces cognitive deficits and improves cerebrovascular function and neuropathology in diabetes-related ADRD (T2DN) rats and TgF344-AD rats. We found that chronic inhibition of sEH with TPPU (1 mg/kg/day in drinking water for 9 weeks) ameliorates cognitive impairment, associated with improving cerebral blood flow autoregulation, enhanced the myogenic response of the middle cerebral artery (MCA) and parenchymal arterioles, reducing amyloid plaques in the cortex and hippocampus, and increasing neuronal counts in the hippocampus in both AD and T2DN rats. EC5026, structurally similar to TPPU, has higher binding potency for recombinant human sEH than for rodent sEH targeted by TPPU. It completed two Phase 1a and one Phase 1b trials with no reported side effects in healthy subjects. This study tested whether EC5026 produces effects similar to TPPU in T2DN rats. Chronic EC5026 treatment (3 mg/kg/day in drinking water for 6 weeks) in T2DN rats did not affect body weight, plasma glucose, or HbA1c levels but significantly improved episodic-like recognition memory, similar to the effects previously observed with TPPU. As assessed by the Novel Object Recognition and Placement Test, the Recognition Index increased from 0.51 ± 0.05 to 0.61 ± 0.06, and the Discrimination Index from 0.01 ± 0.05 to 0.21 ± 0.11 in vehicle-treated vs. EC5026-treated rats (n = 10). The myogenic response of the MCA was impaired in ADRD rats but was normalized with EC5026 treatment. When intraluminal pressure increased from 40 to 180 mmHg, the inner diameter of the MCA increased by 49.32 ± 8.11% in vehicle-treated DM rats (n = 18) and by only 12.81 ± 2.46% in EC5026-treated DM rats (n = 29). These findings suggest that EC5026, like TPPU, restores cognitive function and cerebrovascular integrity in diabetes-related ADRD. Supporting its

therapeutic potential as a human sEH inhibitor for treating vascular contributions to cognitive impairments.

Disclosures: G. Morgan: None. **C. Tang:** None. **S. Wang:** None. **J. Xu:** None. **A. Gregory:** None. **C. Cantwell:** None. **D. Bunn:** None. **R.J. Roman:** None. **F. Fan:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

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Program #/Poster #: PSTR065.16/D59

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

Title: Piezo1 activation improves cerebrovascular function in alzheimer's disease

Authors: *Z. TABRIZI[1], X. LIM[3], S. CHAKRABORTY[1], O. HARRAZ[4], O. BRACKO[2]; [2]Dept. of Biol., [1]Univ. of Miami, Miami, FL; [3]university of vermont, Burlington, VT; [4]Pharmacol., Univ. of Vermont, Burlington, VT

Abstract: Alzheimer’s Disease (AD) is the most common form of dementia. Hallmarks of AD, such as neurodegeneration and cognitive decline, are often preceded by impairment of cerebral blood flow (CBF), typically due to cerebrovascular dysfunction. CBF impairment, particularly in capillaries, the critical sites oxygen and nutrient exchange in response to neuronal activities, may trigger vascular inflammation and disrupt blood flow. Such disruption can alter hemodynamic mechanical forces that are sensed by mechanosensors located on the endothelium. Specifically in brain capillaries, the mechanosensitive Piezo1 ion channel plays a role in mediating calcium transients and regulating CBF. However, whether Piezo1 contributes to AD pathology remains unknown. To investigate this, we utilized pharmacological methods to manipulate endothelial Piezo1 function in 4-month-old 5xFAD mouse model. We employed in vivo multiphoton imaging and measured CBF and capillary stalling following injection with Yoda1, a specific pharmacological activator of Piezo1, baseline, 24 hours post-treatment, and one week following Piezo1 activation. Our findings indicate that modulating Piezo1 activity significantly reduced capillary stalling and improved CBF. Further, Yoda1 treatment rescued functional hyperemia, as measured by laser speckle contrast imaging in 4-month-old 5xFAD mice. Our findings suggest that targeting Piezo1 may offer a novel therapeutic strategy for addressing neurovascular dysfunction in AD.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

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Program #/Poster #: PSTR065.17/D60

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

Support: NIH Grant R01AG074552

Title: Brain vascular differences in two mouse models of Alzheimer's Disease

Authors: *A. F. CITALAN-MADRID[1], D. A. LAWRENCE[3], G. G. MURPHY[2], K. STANGIS[4], E. J. SU[5];
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Abstract: Alzheimer's Disease (AD) is the leading cause of dementia in elderly age. Peripheral vascular etiologies (i.e., hearth failure or hypertension) can remodel the CNS vasculature, compromise the blood brain barrier (BBB), and promote dementia. Mouse models exhibiting hallmark AD pathologies, including amyloid deposition (5xFAD) and hyperphosphorylated tau (PS19) are widely use to study AD phenotypes. Until recently, brain microvasculature remodeling in AD has been considered secondary to neuronal damage and its role has been little studied. Here, we characterize the Aβ-plaque accumulation and microvascular changes in AD mouse brain. Using both male and female 5xFAD and PS19 mice with appropriate controls, we stained the brain microvasculature and implemented the CUBIC-tissue clearing for 3D visualization and analysis. Additionally, we stained β-amyloid plaques to quantify its distribution through different brain regions. We observed amyloid deposition in the brains of 5xFAD mice, and identified the subiculum the most affected area, followed of dentate gyrus (DG) and cortex. Amyloid accumulation was more severe in the 5xFAD females, consistent with previous reports. Interestingly, male 5xFAD mice exhibited increased cortical blood vessel diameter, while 5xFAD females showed elevated vascular density in the subiculum. Although not entirely significant, the hippocampus of female mice showed increased of tortuosity, density and reduction of the average length of blood vessels. In PS19 mice, we identified brain atrophy near the hippocampal region. In addition, we found that while the average diameter of blood vessels was not affected, there was a significant increase in vessel density and tortuosity, and a reduction of the average length of blood vessels in both sexes compared to WT controls. Notably, the piriform cortex of PS19 males showed increased density, tortuosity, and a reduction in length of blood vessels compared to females PS19 or WT controls. These data allow us to conclude that blood vessel remodeling and its damage appear to be determined by the sex and genotype of such transgenic mice. Even more, the degree of blood vessel damage, or the accumulation of amyloid protein seems to be different for each analyzed brain region.

Disclosures: A.F. Citalan-Madrid: None. **D.A. Lawrence:** None. **G.G. Murphy:** None. **K. Stangis:** None. **E.J. Su:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.18/D61

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

Support: NIH GMS 3P20GM121307-02S1
NIH GMS HL149264-01A1

Title: Increased Plasma Cystathionine Beta-Synthase in Human Alzheimer's Disease and Related Dementias (ADRD): Correlation with Cognitive Performance

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Abstract: There is growing evidence that vascular stress is an important contributor to the pathogenesis of Alzheimer's disease (AD). Our recent work suggests links exist between redox-related *disturbances in sulfide metabolism* and measures of cerebrovascular disease and cognitive disturbance in human ADRD. We reported elevated levels of total, acid-labile, and bound plasma sulfides in Alzheimer's disease and related dementias (ADRD) which were associated with cognitive dysfunction, brain atrophy and cerebral microvascular disease. While the increased levels of these sulfide species have now been described in human ADRD, the tissue and enzymatic sources remain unclear, but could provide important insights into the pathomechanisms linking plasma sulfides with disease activity and progression. We therefore tested the hypothesis that elevated levels of enzymatic sulfide sources were associated with increased plasma sulfides and decreased cognitive function. We compared the levels of 3 enzymatic sources of plasma sulfides, cystathionine beta synthase (CBS), cystathionine gamma-lyase (CSE), and 3-mercaptosulfo transferase (3-MST) as well as plasma beta-amyloid in healthy controls (n=29) and individuals who met the criteria for ADRD (ADAS-cog>=17; n=35). We found that plasma CBS was significantly increased in ADRD ($F(3, 60) = 0.732$, $p = 0.396$), while neither MST nor CSE were significantly different across disease groups. Of note, CBS was associated with poorer cognitive performance ($r(63)=.534$, $p<0.0001$). Plasma beta-amyloid was significantly increased in the ADRD group, although not significantly correlated with cognitive function. MST was positively correlated with beta-amyloid levels. CSE was not related to cognitive scores or plasma beta-amyloid. A better understanding of the underlying vascular pathomechanisms and consequences that confer greater risk due to excess sulfide burden may lead to improved detection and treatment of ADRD.

Disclosures: E.A. Disbrow: None. **J. Alexander:** None. **K. Stokes:** None. **C. Kevil:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.19/D62

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

Support: R25HL147665
NIH NHLBI T32HL155022
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Malcolm Feist pre doctoral fellowship from the Center for Cardiovascular Diseases and Sciences to Tashawna Esmond

Title: Plasma levels of redox pathway proteins correlate with cognitive impairment in Alzheimer's Disease and Related Dementias

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Abstract: Introduction: Alzheimer's disease and related dementias (ADRD) are the most common forms of dementia and the 6th leading cause of death worldwide. Vascular dysfunction contributes to the development of cognitive impairment preceding onset of symptoms of ADRD. Early vascular changes are associated with reactive oxygen species production and inflammation. We hypothesized that plasma of people with ADRD would show signs of elevated oxidative stress (increased expression of pro-oxidant enzymes and decreased antioxidant enzyme levels) that correlated with the severity of cognitive impairment as measured by the ADAS-cog score. Methods: Plasma samples were isolated from blood drawn from control and ADRD participants. Dot blot analyses were performed, and blots were probed for antioxidant enzymes (superoxide dismutase 3 (SOD3), catalase (CAT), glutathione peroxidase (GPx), transcription factor Nuclear factor erythroid 2-related factor 2 (Nrf2)), and a subunit of the superoxide-generating enzyme NADPH oxidase (NOX2). Results: The expression levels of NOX2, SOD3, CAT, and Nrf2 were significantly higher in ADRD plasma versus controls. Plasma NOX2, SOD3 and Nrf2 levels positively correlated with ADAS-cog scores, indicative of cognitive impairment. However, no such correlations were observed among the redox proteins. When separated by race, white, but not black, ADRD groups had significant increases in SOD3 and NOX2 as compared to race matched control. In contrast, both white and black ADRD groups exhibited elevated Nrf2 levels vs corresponding controls. Conclusion: Measurements in human plasma of people with ADRD revealed increased NOX2 and antioxidant enzyme levels, with the latter potentially being compensatory responses to oxidative stress. When we assessed race differences in our control and ADRD groups, the direction and magnitude of change for all proteins were largely the same between black and white subjects, although white subjects tended to have a greater increase in NOX2 versus black subjects, which is consistent with past work showing race differences in ADRD pathophysiology.

Disclosures: T. Esmond: None. **C. Kevil:** None. **J. Alexander:** None. **E.A. Disbrow:** None. **K. Stokes:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.20/D63

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Valour Foundation - Rebecca E Barchas MD, DLFAPA University; Professorship in Translational Psychiatry; Morley-Mather University, Hospitals Chair in Neuropsychiatry

Title: A Neuroprotective Role for PGD2/DP1 Signaling in Blood-Brain Barrier Integrity

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Abstract: Neurodegenerative diseases affect millions globally, yet their mechanisms remain poorly understood, leaving therapeutic options severely limited. Emerging research highlights the blood-brain barrier (BBB) as a potential target for neuroprotective therapy, as this structure is compromised early in many neurodegenerative disorders. Notably, obesity and diabetes, established risk factors for neurodegeneration, are also linked to BBB dysfunction. The BBB comprises multiple cell types, including astrocytes, microglia, neurons, pericytes, and microvascular endothelial cells, which constitute a critical regulatory interface for brain health. While peripheral endothelial cells utilize prostaglandin D2 (PGD2) signaling through the DP1 receptor for homeostasis, the role of this pathway in microvascular endothelial cells of the BBB remains unexplored. To address this gap, we exposed 5 week old DP1 knockout (KO) mice, and their wild-type (WT) littermates, to either a high fat diet (HFD) or a standard diet for four weeks. Body weight was measured weekly and blood and tissues were collected after the exposure was complete. HFD-fed DP1 KO mice exhibited significantly elevated total white blood cell and lymphocyte counts compared to HFD-fed WT littermates, indicating heightened peripheral inflammation, a known contributor to BBB compromise. HFD-fed DP1 KO mice also displayed elevated levels of peripheral proteins in the cortex and hippocampus, indicating BBB functional deterioration. Our results suggest that DP1 receptor signaling may play a protective role in preserving BBB integrity during peripheral stressors like HFD-induced inflammation. This implication of the PGD2/DP1 receptor axis in BBB resilience identifies a potentially novel therapeutic avenue to safeguard neurovascular health in the early stages of neurodegenerative disease.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.21/D64

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: GRI1063542

Title: Perivascular Macrophages Mediate Vascular Dysfunction and Cerebral Amyloid Angiopathy in Alzheimer's Disease Models

Authors: *Y. CHIU;
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Abstract: Title: Perivascular Macrophages Mediate Vascular Dysfunction and Cerebral Amyloid Angiopathy in Alzheimer's Disease Models
Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-beta (Aβ) plaque deposition, tau pathology, and cognitive decline. Recent studies highlight the significant role of vascular dysfunction in AD, particularly cerebral amyloid angiopathy (CAA), which disrupts the blood-brain barrier (BBB) and impairs cerebral blood flow. Perivascular macrophages (PVMs), a specialized subset of brain-resident macrophages, play a critical role in both Aβ clearance and neuroinflammation. However, chronic activation of PVMs has been implicated in exacerbating vascular dysfunction and contributing to CAA. This study explores the role of PVMs in driving cerebrovascular pathology in AD models, particularly focusing on their impact on CAA and amyloid accumulation.**Method:** We will utilize two transgenic mouse models of AD, APP/SAA and APP/SwDI, to examine the progression of vascular dysfunction and CAA. These models will be assessed at multiple time points for cognitive function, using behavioral tests, including novel object recognition. Vascular integrity will be evaluated using advanced imaging techniques to assess changes in cerebral blood flow and BBB permeability. To investigate the role of PVMs in vascular damage, we will employ clodronate liposome-mediated macrophage depletion, assessing its effects on both amyloid deposition and cerebrovascular health. Additionally, we will explore the impact of the anti-Aβ antibody on amyloid burden and evaluate potential adverse vascular effects using MRI and histological analyses.
Result: We expect to observe significant vascular dysfunction and CAA in the AD mouse models, with a strong correlation between PVM activation and the progression of cerebrovascular impairment. Depletion of PVMs is anticipated to reduce CAA-associated damage and improve cerebrovascular function. The study will reveal the complex interplay between PVMs, amyloid pathology, and cerebrovascular health in AD.
Conclusion: This study will provide important insights into the role of PVMs in mediating vascular dysfunction and CAA in AD. By elucidating the mechanisms through which PVMs contribute to cerebrovascular pathology, our findings could inform new therapeutic strategies

targeting both amyloid clearance and vascular protection in AD and other neurodegenerative diseases characterized by neuroimmune-vascular interactions.

Disclosures: Y. Chiu: None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.22/E1

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

Support: NIH/NIA RO1 AG065345
Leducq Transatlantic Network of Excellence on Brain Clearance

Title: Laminin regulates perivascular clearance and microvascular function in cerebral small vessel disease

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Abstract: Cerebral small vessel disease (CSVD) affects 11 million Americans and contributes to 45% of dementia cases. Diagnosis of CSVD includes the presence of MRI-visible anomalies such as enlarged perivascular spaces (PVS). The PVS surrounds penetrating arterioles, where it functions as a key entry route of cerebrospinal fluid (CSF) into the brain parenchyma. While growing evidence suggests that enlarged PVSs indicate altered brain clearance, the molecular mechanisms underlying their dysfunction remain unclear. A recent human genome-wide association study linked a hypomorphic mutation in γ-Laminin, a vascular basement membrane protein, to increased PVS burden on MRI. Moreover, in aging and neurodegenerative diseases such as Alzheimer's and cerebral amyloid angiopathy, basement membrane composition, including γ-Laminin, is altered. To investigate γ-Laminin's role in PVS function and cerebrovascular integrity, we used a mural and fibroblast cell-specific knockout mouse model (Pdgfrb-Cre; Lamc1$^{fl/fl}$; Ai14, "PKO"). In a subset of mice, mural cells and fibroblasts were labeled with tdTomato, while astrocytes were labeled with GFP via intravenous AAV (PHP.eB/2-hGFAP-EGFP), allowing visualization of the intervening PVS. In vivo two-photon imaging through thin-skull cranial windows enabled high-resolution visualization of PVS morphology, cerebral microvascular architecture, and blood flow dynamics, complemented by postmortem histology. To our knowledge, this is the first study employing non-invasive, cell-specific labeling to visualize the PVS in mice, avoiding potential artifacts in CSF dynamics caused by cisterna magna tracer injections. We found that γ-Laminin deficiency increases the proportion of visible perivascular spaces in vivo. PKO mice also showed reduced CSF tracer influx after cisterna magna injection and decreased amyloid-beta clearance following

intraparenchymal injection in postmortem analysis. At the capillary level, γ-Laminin deficiency caused vessel dilation and heterogeneous blood flow, characterized by elevated red blood cell flux, blood velocity, and vessel stalling. Pericyte coverage was reduced in PKO mice; however, no direct correlation was observed between pericyte loss and vessel dilation. Finally, vascular alterations coincided with impaired blood-brain barrier integrity. Our findings identify γ-Laminin as a critical regulator of perivascular clearance and microvascular function. This work provides mechanistic insight into CSVD pathophysiology and highlights a potential molecular link between basement membrane remodeling and impaired neurovascular clearance in aging and neurodegeneration.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.23/E2

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

Support: P30 AG086404
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Harvey Family Endowment
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Title: Vascular Integrity and micro/nanoplastics in the human brain

Authors: *E. L. BEARER[1], M. A. GARCIA[2], A. COLLAZO[4], M. J. CAMPEN[5], G. A. ROSENBERG[3];
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Abstract: Vascular pathology contributes to many types of cognitive impairment and neurodegenerative disease and may be imaged by ante-mortem FLAIR MRI. Micro-nanoplastics (MNP) identified by chemical analysis are found in post-mortem brains from healthy individuals (Nihart et al., *Nature Medicine* 2025) and in the choroid plexus of both Alzheimer's and vascular dementias (Bearer et al. 2025). Here we report discovery of abundant MNP in vascular walls of human subcortical white matter in cases of Binswanger's and Alzheimer's disease, and new methodology to image them in histopathology. AD cases were positive for ABeta plaques, p-tau and neuritic plaques (CERAD). In contrast, Binswanger's had diffuse Abeta and little p-tau. Neither disease had TDP43 or synuclein aggregates. In Binswanger's, subcortical white matter

had clusters of CD68-positive macrophages, loss of myelin and collagenosis within adventitia of blood vessels. Both diseases displayed glossy brownish deposits not stained for hemosiderin with Prussian blue near blood vessels. Brain tissue (~5 mg) from regions adjacent to the histopathology blocks from white matter, gray matter and choroid plexus were submitted for chemical analysis. Large amounts of plastics were isolated from these by standard plastics isolation procedures (saponification, centrifugation, and ethanol washes of the pellets, followed by resuspension and separation by pyrGC/MS). At least 6 different plastic polymers were detected by comparison of the GC/MS spectrogram to those of 12 types of pure plastics, the most abundant being polyethylene. Isolated plastic particles were imaged by negative stain EM and confocal laser scanning microscopy. They were found to excite in near ultraviolet light and emit at longer wave lengths across the spectrum. Using this near UV excitation on human brain sections revealed that brownish deposits not staining for hemosiderin had the spectral properties of isolated micro/nanoplastics. We are now mapping the distribution of these fluorescent particles throughout the brain in cases of AD and other dementias. Particles are of different sizes and shapes. Some are aggregated and others diffusely distributed. Excitation-emission spectra, degree of aggregation and size of aggregates differs between locations and cases. Large numbers of fluorescent particles were commonly found in the walls of blood vessels while some particles were randomly distributed throughout white and gray matter in both diseases. We are actively investigating whether micro/nanoplastic locations, aggregation and/or abundance correlate with inflammatory cell responses, blood vessel leakage, ischemia or neuronal cell death.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.24/E3

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

Support: NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program (Award #2014217)

Title: Behavioral changes, increased neural and intrinsic responses and neurovascular coupling in aging APOE4-TREM2 mice as early marker for Late Onset Alzheimer's Disease.

Authors: M. M. OUMOV[1], B. G. SANGANAHALLI[2], P. HERMAN[2], F. HYDER[3], *J. V. VERHAGEN[4,5];
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Abstract: Alzheimer's disease (AD) is an incurable degenerative disease that affects millions worldwide, making early detection crucial for treatment. Many genes have been identified as risk factors for the predominant form, Late Onset AD (LOAD), two of the most impactful being Apoe4 and Trem2 (A4T2). These genes are involved with lipid trafficking, vascular function and inflammation and show age and sex-dependent effects. The MODEL-AD consortium has generated A4T2 mice (JAX #028709) incorporating these 2 humanized mutations. We have reported that these mice show evidence of age-dependent AD-relevant deficits in anxiety, odor-based foraging and spatial memory (bioRxiv 2025.04.20.649712; doi: https://doi.org/10.1101/2025.04.20.649712). We also reported that intrinsic (blood volume, BV) responses to odorants in the olfactory bulb (OB) increased in this A4T2 model with age. Here we report the neurovascular coupling in the OB of aged A4T2 mice. We hypothesize that in early stages of LOAD, neurovascular hyper-responsiveness will be detected due to compensatory efforts sustaining the degenerating tissues.A4T2 mice (JAX #028709) were crossed with mice reporting activity from OB output neurons (mitral/tufted cells, M/TC; Thy1-GCaMP6f). Their offspring were optically imaged for blood volume and calcium responses to odor stimulation of ethyl butyrate and methyl valerate at 0.3, 1, 3% vapor pressure at 10-17mo (13.2±2.8; 5 males, 3 females). Optical window surgeries were performed under isoflurane and mice were imaged under ketamine and dexmedetomidine. BV and Ca responses were quantified as area under the curve, which revealed concentration and odor-dependent responses in the glomeruli and microvessels. Both BV (r^2=0.55) and calcium responses (r^2=0.41) increased with age. Their ratio (coupling) also increased (r^2=0.12). We also report the changes in hemodynamic response function. Neurovascular dysfunction is detectable as both an exacerbated neural and intrinsic responsiveness in the early stages of these LOAD model mice. These preliminary data support vascular hyper-responsiveness as an early marker to LOAD detection that can be assessed with fNIRS and BOLD fMRI.

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Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.25/E4

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

Title: Systems-level, large-scale single-cell RNA-seq analyses reveal identity shifts and lipid-related alterations in human brain capillary endothelial cells in Alzheimer's disease

Authors: *M. ISLAM, L.-H. TSAI;
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Abstract: Blood-brain barrier (BBB) breakdown is implicated early in numerous neurodegenerative diseases. Endothelial cells, due to their localization at capillaries, arteries, and venules, are the first barrier cell type to encounter peripheral changes and challenges from the bloodstream. Here, we investigate the heterogeneity of brain capillary endothelial cells and the transcriptomic and cellular changes they undergo in the context of Alzheimer's disease (AD). Through *in silico* sorting of endothelial cells from single-nucleus RNA sequencing (snRNA-seq) datasets acquired from a cohort of 427 individuals, we delineate vulnerable capillary endothelial subtypes in AD. Moreover, we identify a gene module (endoM2) that is significantly associated with Alzheimer's pathology and APOE4 risk allele, inversely correlated with measures of cognition, and conserved across additional large-scale human snRNA-seq datasets of AD. Biological processes associated with this upregulated endoM2 module include cell migration, immune response, and lipid transport. To establish a gene-module-to-phenotype connection, we leverage human induced pluripotent stem cells (iPSCs) to differentiate brain microvascular endothelial cells (BMECs) and test various stimulation conditions informed by the identified GO terms in endoM2. We find a cytokine treatment leads to cellular elongation, increased lipid uptake and lipid droplet formation, and tight junction losses. We posit that BMECs undergo inflammation-induced changes in Alzheimer's disease, exacerbated by *APOE4* risk gene status, that suggest a loss of cellular identity of endothelial cells and result in loss of BBB integrity. Bulk sequencing of the cytokine-treated iPSC-derived BMECs from two isogenic pairs reveals that genes associated with cell migration are perturbed. From a targeted drug screen to inhibit top altered genes, we discover an exciting small molecule that limits cellular elongation, decreases lipid droplet accumulation, and reduces tight junction losses, validated in both 2-D cell monoculture and 3-D *in vitro* BBB co-culture. Our work motivates targeted interventions to prevent cerebrovascular dysfunction in the face of peripheral inflammation.

Disclosures: M. Islam: None. **L. Tsai:** None.

Poster

PSTR065: Vasculature, BBB, and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR065.26/E5

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: R00AG080034

Title: Intercellular interactions between perivascular fibroblasts an macrophages in the brain: Implications for vascular homeostasis in health and cerebral amyloid angiopathy

Authors: *L. MUNOZ[1], M. SOSA[2], A. Y. SHIH[3], S. GRUBB[4], S. BONNEY[5];
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Abstract: Perivascular spaces (PVS) are fluid-filled compartments surrounding blood vessels in the brain, acting as niches for various cell types, including perivascular fibroblasts (PVFs) and perivascular macrophages (PVMs). While the role of PVMs is increasingly understood, PVFs, a specialized population of brain fibroblasts, remain poorly characterized. Given that PVSs are key sites of amyloid-β (Aβ) accumulation in cerebral amyloid angiopathy (CAA), commonly seen in Alzheimer's disease (AD), PVFs may play a critical role in vascular health and be directly affected by Aβ pathology. In the tgSwDI AD mouse model, that has higher prevalence of CAA, we observed early reductions in PVF populations, at the onset of parenchymal Aβ and prior to CAA. This also coincides with morphological changes in PVMs, indicating significant changes occur in the PVS niche prior to the vascular Aβ accumulation. To study PVS microenvironment and interactions occurring between PVFs and PVMs in the healthy brain, we used a 3D-electron microscopy (EM) dataset from the Machine Intelligence from Cortical Networks (MICrONS) program, which includes large-scale EM reconstructions from the mouse visual cortex. We mapped out PVFs and PVMs on arterioles and venules in the $1mm^3$ EM dataset and found there are 303 PVF and 156 PVMs. PVFs were associated with PVMs in about 70% of cases, while PVMs were associated with PVFs in 86% of cases. They exhibited direct physical interactions, including soma-soma (30%), soma-process (38%), and process-process (32%) contacts. 3D reconstruction of a PVF-PVM pair revealed their intricate and complex interactions along their interface. We analyzed a subset of the PVF-PVM interactions and in approximately 78% of cases, the cells shared vesicular structures at their interfaces, with apparent directional transfer from PVFs to PVMs observed in 83% of these interactions. While the identity of these PVF-vesicles is unknown, it is possible they are related to lysosomal processes known to macrophages. Together, our findings highlight a potentially critical role of PVF-PVM interactions in maintaining vascular homeostasis. The disruption of this interface may represent an early hallmark of AD progression. The early loss of PVFs observed in AD models suggests impairments in vesicle-trafficking at the PVF-PVM interface. This could compromise perivascular cell function, PVS homeostasis, and contribute to the vascular pathology observed in CAA.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.01/E6

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

Support: Grant-in-Aid for Scientific Research (B), Japan Society for the Promotion of Science (JSPS)
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Title: A curcumin-derived compound ShigaY-51 attenuates amyloid-β oligomer-induced cytotoxicity in SH-SY5Y cells

Authors: *S. TAKAHATA[1], T. KATO[1], D. YANAGISAWA[1], R. RAZALI[1], J.-P. BELLIER[3,2], I. TOOYAMA[1];
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Abstract: Amyloid-β (Aβ) oligomers are widely recognized as neurotoxic entities involved in the pathogenesis of Alzheimer's disease. ShigaY-51 (SY51) is a novel fluorinated small molecule derived from curcumin that selectively binds to toxic Aβ oligomers and enables their in vivo detection by fluorine-19 magnetic resonance imaging, as demonstrated in the APP/PS1 mouse model of Alzheimer's disease. In the present study, we examined the protective effect of SY51 against Aβ oligomer-induced cytotoxicity in SH-SY5Y neuroblastoma cells. $A\beta_{1-42}$ peptides were pretreated with HFIP to eliminate structural heterogeneity, then dissolved in DMSO and diluted in PBS with 0.2% SDS. After incubation at 37°C, oligomers were formed and confirmed by western blotting using anti-Aβ polyclonal antibody. SH-SY5Y cells were exposed to Aβ oligomers at concentrations ranging from 0.1 to 3.0 μM, and cell viability was assessed using the MTT assay. A concentration-dependent decrease in cell viability was observed, with cell viability reduced to approximately 50% at 0.1 μM Aβ oligomers. Co-treatment with SY51 significantly improved cell viability, most notably at 0.3 μM Aβ ($P < 0.05$). These results suggest that SY51 effectively attenuates Aβ oligomer-induced cytotoxicity in vitro. Further investigations are in progress to assess its therapeutic efficacy in a mouse model of Alzheimer's disease.

Disclosures: S. Takahata: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); I am the founder and owner of Medpreneur Inc. The company did not provide any funding for this research.. **T. Kato:** None. **D. Yanagisawa:** None. **R. Razali:** None. **J. Bellier:** None. **I. Tooyama:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.02/Web Only

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

Support: NIH Grant R16NS140308

Title: Dual Sigma Receptor Modulator Regulates IRE1-Mediated Unfolded Protein Response to Amyloid-beta in SH-SY5Y Cells

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Abstract: Sigma receptors present a novel avenue of therapeutic targeting of Alzheimer's Disease, capable of impacting Aβ toxicity, neuroinflammation, and apoptosis. These actions may occur through an association with unfolded protein response (UPR) component, inositol requiring enzyme 1 (IRE1). IRE1 is activated by Aβ in the endoplasmic reticulum (ER) and may signal for cell survival with low levels of ER stress through X-box binding protein 1 (XBP1) splicing in the adaptive pathway. Alternatively, IRE1 may signal apoptosis under high levels of ER stress (e.g., excess Aβ) through the terminal pathway by activating thioredoxin-interacting protein (TXNIP), c-Jun N-terminal kinase (JNK), and nuclear factor kappa B (NFκB). To investigate sigma receptor involvement in IRE1 mediated UPR, the novel sigma-1 and sigma-2 dual modulator, BBZI (0, 0.01, 0.1, and 1.0 μM), was evaluated the cascade of events mRNA expression changes of genes involved in the adaptive and terminal pathways of the UPR in SH-SY5Y cells treated with Aβ (1.0 μM). BBZI significantly impacted Aβ induced changes in mRNA expression of XBP1 ($p < 0.0001$), IRE1 ($p < 0.0001$), TXNIP ($p < 0.0001$), JNK ($p < 0.0001$), and NFKB ($p < 0.0001$). BBZI (0.1 μM) co-treatment with Aβ (1 μM) upregulated mRNA expression of the adaptive pathway through XBP1 ($p < 0.0001$) and reduced IRE1 mRNA expression overactivation ($p < 0.001$) but also downregulated terminal pathway components including TXNIP ($p = 0.003$), JNK ($p = 0.003$) and NFκB ($p = 0.003$) when compared to Aβ alone. BBZI pretreatment also impacted neuronal viability in response to Aβ ($p < 0.0001$). Neuronal viability was assessed using the MTT test and increased with 0.1 μM BBZI and Aβ when compared to Aβ alone ($p = 0.0023$). Taken together, the data suggests sigma receptor modulator BBZI regulates the IRE1 mediated UPR by increasing the adaptive pathway and decreasing the terminal pathway in response to Aβ as a possible mechanism for maintaining neuronal viability.

Disclosures: A. Loraine: None. **K.E. Sandoval:** None. **M. Crider:** None. **K.A. Witt:** Other; Commercial Interest.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.03/E7

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

Support: Jane and Aatos Erkko Foundation

Title: Pp2a activation by prolyl oligopeptidase ligands reduces tau aggregation and neuritic plaques in an in vitro alzheimer's disease model

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Abstract: The key pathological features in the Alzheimer's disease (AD) includes the formation of amyloid plaques and neurofibrillary tangles rich of hyperphoshorylated Tau. Current therapies have very limited efficacy on disease-progression, and therefore, novel disease-modifying therapies are urgently needed. One early and very specific feature in AD is formation neuritic plaques. Endosomes, such as autophagosomes, get trapped and form the neuritic plaques in the axons while the neuronal cell tries to export these aggregated neurotoxic proteins. The hypothesis is that neuritic plaques damage axons, leading to synaptic and later on neuronal death. We have earlier revealed that the small-molecular ligands for prolyl oligopeptidase (PREP) decreased tau aggregation and enhanced autophagy by activating protein phosphatase 2A (PP2A). In this study, we wanted to assess if PREP ligands can decrease the formation of neuritic plaques and reduce Tau aggregation by improving axonal transport in and *in vitro* AD model. The pathology and neuritic plaques were induced in mouse primary cortical neurons by using amyloid β oligomers (1-42) (AβOs) with 10, 50, 100 nM concentrations, and 24 and 48 h time points. The primary neurons were simultaneously incubated with 10 uM HUP-46, a novel PREP ligand. The impact of AβOs and HUP-46 on markers of neuritic plaques (LC3B and LAMP1 for autophagosomes and lysosomal structures, Tau5 for total Tau and AT8 for phosphorylated Tau) were studied by using Western blot and confocal imaging. 10 μM HUP 46 increased the LC3BII/LC3BI ratio and decreased LAMP1, suggesting that HUP-46 reversed the blockage of autophagosomes and lysosomes in 50 nM AβOs-treated neurons. Furthermore, HUP 46 significantly decreased AT8 and Tau 5 levels in the neurons exposed to 50 nM AβOs in Western blot. Meanwhile, the confocal imaging showed that HUP- 46 reduced the LC3B accumulation in the axons and decreased the colocalization between LC3B and AT8, indicating that the PREP ligands decrease the neuritic plaques induced by AβOs. Our results indicated that a novel PREP ligand, HUP-46, reduced the formation of neuritic plaques in an AD cellular model. As we have earlier shown a disease-modifying effect by PREP ligands in Tau transgenic mice, our current results may expand the usability of PREP ligands also as AD therapy.

Disclosures: B. Fastré: A. Employment/Salary (full or part-time): University of Helsinki. **H. Cui:** A. Employment/Salary (full or part-time): University of Helsinki. **T.T. Myöhänen:** A. Employment/Salary (full or part-time): University of Helsinki.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.04/E8

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

Support: Parul University Intramural Research Grant RDC/IMSL/229

Title: Prenylated quinoline carboxylic acid as a promising anti Alzheimer agent targeting neuroinflammation and autophagy

Authors: *N. PATIL[1], M. JAIN[2];
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Abstract: According to the WHO, in 2021, there were around 57 million cases of dementia worldwide, of which about 60% to 70% were due to AD. It is anticipated that the number of people over 65 will increase from 76 million in 2020 to 117 million by 2050, which is the most vulnerable population and prone to AD. Among the multiple causes of AD progression, most drugs target amyloid processing, tau pathology, imbalances in neurotransmitters, problems with synapses, and oxidative stress. Apart from these traditional factors, the other two pathways are dysregulated in AD: Neuroinflammation and Autophagy. It is being reported that Pro-inflammatory chemokines promote cognitive decline, whereas autophagy dysfunctions cause protein toxicity and synaptic loss. Even as per the 2024 AD drug pipeline (CADRO, 2024), more therapies are needed to focus on the cellular and immune aspects of the disease, and it is being reported that fewer than 20% of active trials address inflammation or autophagy. To address this limitation, we explored PQA-18, with known IL-13 inhibition and immunosuppressive effects. Network pharmacology, docking (COX-2: −9.2 kcal/mol; PAK2: −7.6 kcal/mol), and 100 ns MD simulations confirmed its stable dual targeting of neuroinflammation and cytoskeletal regulators. For autophagy experimental validation, we use D. discoideum, and for anti-neuroinflammatory activity, we use the SH-SY5Y/HMC3 co-culture model. In D. discoideum, PQA18 (IC50 =14.5 nM) induced a reduction of the growth rate by ~22%, a delay in development by 4 hours, and morphological aberrations such as multi-tipped structures and longer fruiting body stalks, autophagy impairment. We have analyzed the increase in expression of autophagy genes such as atg5, atg8, atg9, atg18, and a decrease in atg1, apart from autophagic flux was studied by GFT-TKT cleavage assay. Based on this data, it was observed that PQA-18 modulates autophagy activity by inducing late autophagy genes. To study the neuroprotective and anti-inflammatory potential of PQA-18, we used the SH-SY5Y/HMC3 co-culture model without $A\beta_{42}$ stress. AD markers reported a lower alteration in Tau phosphorylation went down, suggesting that the cytoskeleton remained stable. In addition, the presence of neuroinflammation markers indicated that levels of BDNF were upregulated, whereas those of TNF-α, COX-2, ICAM1, GFAP, and Iba1 were downregulated, suggesting there was less glial activity and more homeostatic behavior in microglia. These findings suggest that PQA-18 could protect nerve cells by reducing slight inflammation, which may help to suppress the progression of AD.

Disclosures: N. Patil: None. **M. Jain:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.05/E9

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

Title: An unbiased phenotypic screening approach utilizing Cell Painting technology identifies disease specific morphological clusters in iPSC-derived neuronal cells harboring mutations associated with Alzheimer's disease

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Abstract: High attrition rates remain a significant challenge in CNS drug development pipelines, underscoring the urgent need for in vitro model systems with -improved predictive -accuracy and translational relevance to human disease. Since the original publication of the Cell Painting assay protocol by Carpenter et al. in 2016, this unbiased phenotypic screening approach has been widely adopted to uncover novel insights into disease etiology, mechanisms of action and toxicity predictions. At Charles River, we have leveraged this innovative assay to refine de-risking strategies in drug development, to explore underlying cellular disease mechanisms during repurposing screens and to enhance the translatability of disease models. In this study, we compared ioGlutamatergic neurons harboring a homozygous MAPT P301S mutation with wild-type isogenic control neurons. We used a modified version of the traditional Cell Painting protocol, substituting the mitochondrial dye with a β-III tubulin antibody to enhance neural network visualization and structural mapping. Data analysis was performed in alignment with international standards established and published by the JUMP Cell Painting Consortium. Principal Component Analysis (PCA) demonstrated a robust separation between the morphological profiles of wild-type (WT) and Alzheimer's disease (AD) cells, confirming the presence of phenotypically distinct signatures. To further assess disease relevance, cells were exposed to increasing concentrations of β-amyloid (Aβ) oligomers, mimicking the pathological accumulation known to drive neurodegeneration in Alzheimer's disease. We previously reported that such exposure induces neurodegenerative effects, as evidenced by high content assessments and increased neurofilament light chain (NfL) release. In this study, PCA plots revealed a dose-dependent phenotypic shift in the same direction, relative to the untreated cluster position, indicating a consistent and progressive cellular response to Aβ toxicity. Deeper analysis of the features contributing to the cluster separation highlighted alterations primarily related to cellular granularity. Taken together, these preliminary results support the validity and strength of unbiased phenotypic screening in validating disease-relevant cellular models. This approach holds promise for future applications such as compound screening, ultimately aiming to advance the development of effective therapies for Alzheimer's disease.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR066.06/E10

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

Support: NIH MH106640
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VA BRAVE

Title: Neuroprotective effects of the neurotrophic factors carbamoylated erythropoietin and insulin-like growth factor-1 in Alzheimer's disease

Authors: *M. J. ROTHSCHADL, M. SATHYANESAN, S. S. NEWTON;
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Abstract: Cognitive functioning impacts an individual's quality of life, with cognitive decline being a major symptom of Alzheimer's disease (AD). Neurotrophic factors can modulate neuronal survival, structure, and function, impacting cognition, and numerous neurotrophic factors show decreased expression as AD progresses. Previous research has shown that individual neurotrophic factors can reduce amyloid load and improve cognitive functioning in mouse models of AD, but studies have not examined whether combining two neurotrophic factors can enhance these benefits. There are also a lack of studies examining the neuroprotective properties of neurotrophic factors, which will be important for developing newer neurotrophic factor-based therapeutics. In the present study, we investigate the neuroprotective potential of combining two neurotrophic factors, carbamoylated erythropoietin (CEPO) and insulin-like growth factor-1 (IGF-1), in AD. To do this work, male and female 5xFAD mice bred from both maternal and paternal origin were intraperitoneally injected with CEPO, IGF-1, CEPO+IGF-1, Galantamine, or vehicle starting at two months of age, before cognitive deficits are present, until nine months of age. Mouse behavior was analyzed using the open field and Morris Water Maze (MWM) tests at six and nine months of age to assess learning and memory. We found that male 5xFAD mice showed behavioral deficits in the MWM starting at six months of age, and that CEPO and IGF-1 treatments, but not CEPO+IGF-1 or Galantamine, could protect against those deficits. At nine months, the neurotrophic factor-treated male 5xFAD mice showed sustained improvement in the MWM test compared to the untreated 5xFAD mice. The female 5xFAD mice did not show behavioral deficits in the MWM until nine months. At this age, neither the neurotrophic factor- or Galantamine-treated female 5xFAD mice showed significantly different behavioral performance in the MWM test, indicating that these neurotrophic factors were ineffective at protecting against AD progression in female 5xFAD mice. No differences between treatment groups were seen in the open field test. The brains were then removed to perform a correlative neuroanatomical analysis of amyloid load. These results demonstrate a clear sex-specific difference in the neuroprotective effects of CEPO and IGF-1 in protecting against cognitive decline, with these neurotrophic factors being effective in male but not female mice. Our data also suggest that combining two neurotrophic factors may not be more effective than their individual treatment; however, more studies looking at different dosing and timing regimens should be pursued in the future.

Disclosures: M.J. Rothschadl: None. **M. Sathyanesan:** None. **S.S. Newton:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR066.07/E11

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

Support: NIH R01 AG071859
NIH R01 AG073826

Title: The combination of all-trans retinoic acid and vorinostat protects neurons from oxidative stress via the Nrf2-ARE pathway

Authors: *J. J. LAWRENCE[1,2,3,4,5], A. HINDLE[1,3], A. BAKER[1,3,6], S. SMITH[1,3,7], S. SINGH[4,8], C. BOSE[1,3];
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Abstract: Oxidative stress (OS) and mitochondrial dysfunction, due to excess accumulation of reactive oxygen species, are key features of Alzheimer's disease (AD). Diet-derived antioxidants, such as all-trans retinoic acid (ATRA) and OS-responsive transcription factors, such as Nrf2, support exogenous and endogenous antioxidant defenses in neurons. These antioxidant defenses fail in AD. AD also disrupts histone deacetylation (HDAC)/acetylation homeostasis, leading to epigenetic suppression of gene transcription. Here, we used mouse hippocampal HT22 cells *in vitro* to examine whether the HDAC inhibitor vorinostat acts synergistically with ATRA to protect neuronal cells from OS. HT22 cells were pre-treated with ATRA (5μM) and/or vorinostat (VOR; 0.5μM). After 24h, cells were challenged with 50μM H_2O_2 for 24h. Lipid peroxidation (LP), mitochondrial function, DNA fragmentation, apoptosis, Nrf2 expression, and downstream Nrf2 targets were examined via ELISAs, comet and TUNEL assays, flow cytometry, immunocytochemistry, and Western blots. H_2O_2-induced LP, as measured by 4-HNE protein adducts, was attenuated by ATRA and/or VOR pretreatments ($p<0.001$). In addition, as measured by ratiometric LP sensor, confirmed that ATRA and/or VOR attenuated H_2O_2-induced LP ($p<0.0001$, Holm-Sidak). Regarding mitochondrial function, ATRA increased ATP levels alone ($p<0.0001$) and restored H_2O_2-induced reductions in ATP levels ($p<0.05$). As observed by Mitotracker staining, mitochondrial morphology was improved after pretreatment with ATRA and/or VOR. In comet assays, H_2O_2induced DNA fragmentation (94.8±4.7% of DNA in comet tails, $p<0.0001$). However, ATRA and/or VOR pretreatments dramatically decreased DNA fragmentation (9.4±0.3%-17.1±0.61%, $p<0.0001$). Similarly, H_2O_2increased the percentage of TUNEL-positive cells ($p>0.0001$), which were reduced by ATRA and/or VOR pretreatments ($p<0.0001$). Apoptosis analysis revealed similar results. Regarding Nrf2, H_2O_2 induced the translocation of Nrf2 to the nucleus over 12-16 hours; however, nuclear translocation of Nrf2 was reduced by ATRA and/or VOR. Finally, we examined the activities of Nrf2 targets. Initial experiments suggest that the combination of ATRA+VOR has distinct effects compared to VOR or ATRA alone, which is supported in Western blots. In conclusion, our in vitro studies suggest that ATRA and VOR protect neuronal

cells from OS via a crucial mechanism to remove excess 4-HNE in neuronal cells. Our results indicate that VOR acts independently and synergistically with ATRA as an antioxidant. These findings have important implications for OS in neurodegenerative diseases such as Alzheimer's disease.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.08/E12

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

Title: Neurotrophic factor-α1/ Carboxypeptidase E gene therapy and CPE mimetics in treatment of Alzheimer's Disease

Authors: *N. GANJUR[1], X. YANG[2], L. XIAO[2], J.-Y. LEE[3], F. MOGHADAM[4], P. SHARMA[5], B. STOLTZ[4], W. GODDARD[4], Y. LOH[2];
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Abstract: Alzheimer's Disease (AD), the most prevalent neurodegenerative disorder, affects nearly 7 million individuals in the United States. With no effective treatment currently available, AD remains a pressing and unmet medical challenge. We carried out gene therapy with bilateral hippocampal injection of AAV- Neurotrophic factor-α1/ Carboxypeptidase E (NF-α1/CPE) and showed that it reversed cognitive deficits (Water maze and Novel Object Recognition tests), neurodegeneration, downregulated expression of characteristic pathological proteins APP and tau and increased pro-survival protein Bcl2 in 3xTg AD mouse model. In addition, deficits in autophagy and synaptogenesis were rescued by AAV- NF-α1/CPE, indicating strong therapeutic effects of NF-α1/CPE in AD mice. Since direct injection of AAV- NF-α1/CPE into brain is very invasive in a clinical setting, we then designed small molecule CPE mimetics as an alternative therapy. We have previously shown that CPE acts by binding to the HT1E receptor (HTR1E) to activate the ERK-BCL2 pathway. After screening over 6 million compounds, a small-molecule mimetic of CPE, Z124 was identified. Z124 effectively mimicked the effects of CPE, prompting us to develop 10 structural analogs of it. Among the synthesized analogs, two lead candidates, R9 and R10, exhibited strong neuroprotective activity. These compounds demonstrated high binding affinity to 5-HTR1E (Kd = 1.38 ± 0.2 nM for R9 and 2.1 ± 0.2 nM for R10), significantly exceeding that of human WT-CPE (Kd = 26.5 ± 5.6 nM). We used functional assays, including Dynamic Mass Redistribution, LDH cytotoxicity, luciferase reporter binding, and Western blot analysis, to confirm their selective engagement with 5-HTR1E and the activation of the downstream ERK-BCL2 pathway. These effects were initially validated in

HEK293 cells that were stably transfected with the 5-HT1E receptor. We subsequently tested the compounds in primary human neurons. R9 and R10 were found to protect neurons from oxidative stress in human neurons and HEK293 cells, as well as the mouse hippocampal cell line HT22. We determined that both compounds effectively activated the ERK signaling pathway and upregulated the expression of the pro-survival protein BCL2. This indicated that the compounds could enhance cell survival through multiple mechanisms. Our findings highlight R9 and R10 as promising candidates that replicate CPE-mediated neuroprotection and represent a novel therapeutic approach for Alzheimer's Disease. Future experiments will aim to assess whether these compounds, when applied through nasal instillation, could affect reversing AD pathology in AD mouse models.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.09/E13

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

Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)

Title: Cortical delivery of carboxypeptidase E gene protects against neuroinflammation and neurodegeneration after mild traumatic brain injury in mice

Authors: A. KHANAM[1], L. XIAO[3], X. YANG[4], D. ABEBE[2], *Y. LOH[5];
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Abstract: Concussion is a form of mild traumatic brain injury (mTBI) and half the world's population suffer mTBI one or more times in their lifetime. MTBI can trigger a cascade of damage despite the mild symptoms, and repeated mTBI can render neurons vulnerable to degeneration. Repeated mTBI can cause death and disability among all ages. Hippocampal delivery of adeno-associated virus (AAV) carrying the Carboxypeptidase E (Neurotrophic factor-alpha1) gene has been reported to exert neuroprotective function in the brain of Alzheimer Disease mice, reversing neurodegeneration and inflammation by increasing mitochondrial pro-survival protein Bcl2, and decreasing pro-inflammatory protein Card14, respectively. Here, we investigated whether pre-delivery of AAV-CPE into the cortex prior to mTBI can ameliorate neuroinflammation and neurodegeneration. Mature wild-type and Atf3-Cre::Snap25-eGFP mice

received bilateral stereotaxic injection of either AAV-mCherry or AAV-CPE in ±1.3 mm lateral from midline of bregma and 14 days later received mTBI in -2 mm lateral from midline of bregma. Wild-type mice were euthanized after 4 days and 21 days post mTBI, and Atf3-Cre::Snap25-eGFP mice were euthanized after 21days post mTBI. Brain tissues were collected, fixed, sectioned, and sections used for immunostaining. In the wild-type mice, the degenerating neurons in the brain sections were stained by amino-Cupric-Silver method and numbers were counted. In Atf3-Cre::Snap25-eGFP mice, numbers of Atf3+ and Iba1+ cells were counted by immunofluorescence staining of brain sections. In AAV-CPE injected wild-type mice, significantly fewer numbers of degenerating neurons were observed in ipsilateral cortex in 4 days and 21 days post mTBI compared to ipsilateral cortex of AAV-mCherry injected mice. Then, the neuroprotective effect of pre-delivered AAV-CPE 14 days prior to mTBI were tested in Atf3-Cre::Snap25-eGFP mice. These mice co-express eGFP reporter when expressing injury induced stress associated gene Atf3 after mTBI. In these mice, AAV-CPE injection reduced the numbers of Atf3+ green neurons and Iba1+ activated microglia significantly in the ipsilateral cortex in 21 days post mTBI compared to ipsilateral cortex of mice that received AAV-mCherry injection. These findings indicate that the pre-delivery of AAV-CPE gene provides both short-term and long-term neuroprotection in mice after mTBI. These results suggest that AAV-CPE gene delivery to the brain could be a potential therapy to protect against neurodegeneration and to reduce neuroinflammation after mTBI. Future studies are needed to confirm neuroprotective effects of AAV-CPE delivered immediately after mTBI.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.10/E14

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

Support: France 2030 initiative

Title: High-throughput brain-on-chip platform for accelerating neurodegenerative drug discovery

Authors: *N. VIOLLE, V. TALLANDIER, L. DIER, S. KRIDI, A. ALLOUCHE, J. COLIN; ETAP-Lab, Vandoeuvre-lès-Nancy, France

Abstract: Synaptic dysfunction and loss, followed by neurite degeneration are among the earliest and most critical events in neurodegenerative diseases (NDD) such as Alzheimer’s disease (AD). These early pathological changes are strongly linked to cognitive decline and remain underexploited as therapeutic targets. Numerous drug candidates have failed in clinical trials due to late stage targeting and the poor translational value of current preclinical models.

Therefore, there is a pressing need for predictive, high-throughput platforms that replicate early-stage neurodegeneration and support drug discovery.
We developed Brain-on-Chip (BoC) systems combining standard 2D cultures exposed to disease-relevant neurotoxins: amyloid-beta oligomers (AβO) and tau oligomers (TauO) and compartmentalized microfluidic devices to model the early stages of AD. In addition to primary cortical neurons, we incorporated human induced pluripotent stem cell-derived neurons to enhance translational relevance. Here, we also report progress in developing high-throughput *in vitro* systems using a high-content imaging system and automated analyses.
To investigate mechanisms of neurotoxicity, we labeled AβO to track their axonal uptake, intracellular transport, and nuclear entry. Our findings demonstrate that: 1) AβO and TauO significantly impair axonal integrity and induce neurodegeneration; 2) Compartmentalized microfluidic models enable targeted manipulation and analysis of synapses and axons, supporting more nuanced investigation of the early stages of AD; 3) Axonal exposure to labeled AβO reveals their active axonal transport, with subsequent accumulation observed in the neuronal nucleus and; 4) High-content imaging methods accelerate the investigation of early neurodegenerative mechanisms and the screening of drug candidates.
These investigations aim to uncover critical steps in the pathogenic process, enhancing our ability to target early mechanisms of NDD progression and increasing the predictive power of BoC platforms for drug discovery. While 2D cultures confirmed synaptotoxicity and neurite degeneration, microfluidic devices revealed compartment-specific vulnerabilities by targeting neurotoxins to somatic, axonal, or synaptic regions. In conclusion, the ability to isolate and study these regions independently provides a powerful tool to deepen our understanding of NDD. These high-throughput and physiologically relevant models have strong potential to uncover early-stage disease processes and accelerate the discovery of novel therapeutic candidates for AD and related disorders.

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Poster

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Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.11/E15

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

Support: NIH Grant AG084187
Dementia Alliance of North Carolina

Title: Investigating CRISPR-Cas13d-mediated tau depletion as a therapeutic strategy for Alzheimer's disease

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Abstract: Tau pathology is a hallmark of several neurodegenerative diseases, including Alzheimer's disease (AD) and other tauopathies. AD is the most common neurodegenerative disorder, affecting over 7 million Americans, and is the 7th leading cause of death. Accumulation of tau strongly correlates with cognitive decline in AD patients. Despite this, no commercially available tau-targeting therapies exist to date, presenting a pressing need for new treatment options. Notably, tau depletion is well tolerated and alleviates AD phenotypes in mouse and human induced pluripotent stem cell (hiPSC) models even in the presence of amyloid beta (Aβ), suggesting tau is a critical mediator of Aβ toxicity. To achieve targeted tau depletion, we engineered a CRISPR-Cas13d system designed to specifically target and cleave MAPT mRNA. Optimal MAPT-targeting guide sequences were predicted using a computational algorithm. 25 top MAPT-targeting gRNA candidates were produced into lentiviral vectors and delivered to SH-SY5Y cells for screening. Fourteen days post-transduction, cell lysates were harvested for western blot. Top-performing candidates achieving >70% tau knockdown were then produced with the high-fidelity Cas13d variant for increased target specificity and packaged into AAV. Delivery of AAV-CRISPR-Cas13d constructs effectively reduced MAPT in both differentiated SH-SY5Y cells and primary neurons from humanized tau (hTau) mice overexpressing WT neuron tau in the absence of mouse tau. Ongoing work aims to evaluate tau pathology and cell viability after delivery of gRNA candidates to identify the most effective guide. For in vivo validation, AAV-CRISPR-Cas13d will be administered intravenously to hTau mice, which exhibit tau aggregation, cognitive impairment, and neuronal loss. We will assess tau depletion and behavioral outcomes following treatment at either early (preventative) or late (therapeutic) stages of disease progression. Our findings thus far support the feasibility of CRISPR-based MAPT targeting as a non-invasive and long-lasting gene therapy approach for AD and related tauopathies.

Disclosures: J. Lanfersieck: None. **G. Fragola:** None. **T.J. Cohen:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.12/E16

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

Title: Drug screening for upregulation of BDNF expression revealed novel drugs and drug target interactions in vitro.

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Abstract: Brain derived neurotrophic factor (BDNF) plays a critical role in the survival, growth, and synaptic plasticity of neurons that are essential for learning and memory formation. Deficiency of BDNF leads to neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. This study aims to identify drugs that upregulate BDNF expression, thereby supporting neuronal growth and survival. First, we established a cellular read-out system that allows us to distinguish drugs capable of stimulating BDNF promoter activity. To this end, we engineered U87-MG human glioblastoma cells to stably express a construct of human BDNF promoter IV-Luciferase 2, followed by mCherry and neomycin selection markers in a lentiviral vector. Using this cell line, we screened drugs from the NCI Structure Diversity VII library, obtained from the Developmental Therapeutics Program, and manually tested this compound library to find drugs that can upregulate BDNF promoter activity. Amongst 1581 compounds tested, we identified 125 molecules that significantly upregulated BDNF promoter activity when compared to Riluzole, a drug used to treat amyotrophic lateral sclerosis (ALS) and known to upregulate BDNF expression. The structures of the 125 positive compounds from the NCI library were submitted to a machine learning model to identify structural relationships that can be used to determine the drug targets. We selected a cluster of 7 compounds with similar structures and strong activities to upregulate BDNF expression. Interestingly, our docking predictions indicated that each of the 7 structurally related compounds can interact with multiple targets, and that each of these targets can bind multiple compounds of the cluster. The EC_{50} values of these compounds to activate the BDNF promoter ranged from 172 nM to 4.8 μM. We then tested the activity of these 7 compounds to affect one of the predicted shared target proteins, soluble epoxide hydrolase (sEH). Interestingly, TPPU, a known sEH inhibitor, is structurally related to the 7 compounds. This indicates that sEH likely plays a role in the regulation of BDNF promoter activity and can be targeted with novel drugs identified in this study. As a functional assay, we found that some of the identified compounds promoted neurite outgrowth in SH-SY5Y cells *in vitro,* when drugs were added to the media in addition to retinoic acid. The extent of neurite outgrowth positively correlated with BDNF expression level upregulated by the drugs in the SH-SY5Y. We will further explore the functional mechanism through which these 7 compounds upregulate BDNF expression cooperatively, and therapeutic significance of these drugs.

Disclosures: M.S. Anekoji: None. **V. Koznetsova:** None. **B. Felding:** None. **I. Tsigelny:** None. **F. Lin:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.13/E17

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

Support: IIT Kharagpur (Grant No. IIT/SRIC/FSRGI2023/17)

Title: Dimethyl-2-oxoglutarate protects against glut inhibitor-induced neurotoxicity in SH-SY5Y cells: insights into alzheimer's disease

Authors: *A. GHOSH[1], A. CHAUHAN[2], A. BIR[4], S. CHAKRABARTI[5], S. CHAKRABARTI[3];
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Abstract: The current understanding of Alzheimer's disease (AD) pathogenesis places significant emphasis on impaired cerebral glucose utilization. Reduced glucose metabolism, coupled with decreased levels of glucose transporters, is recognized as a hallmark feature of AD. To explore the role of glucose transporters in AD pathogenesis, we observed that the GLUT inhibitor WZB117 caused a significant loss of cell viability in SH-SY5Y neuroblastoma cells along with a marked increase in reactive oxygen species (ROS) production in our earlier study. Extending this, we aimed to investigate whether another pan-GLUT inhibitor, DRB18, could similarly induce neuronal death through enhanced ROS generation. We observed that DRB18 induced a dose-dependent increase in cell death, increased ROS production, mitochondrial membrane depolarization and a depletion of cellular ATP content in SH-SY5Y cells. To restore the damage, we explored molecules like novel lipid-soluble antioxidants (ferrostatin-1 and liproxstatin-1), the water-soluble antioxidant N-acetylcysteine, and alternative metabolic fuels (pyruvate, glutamate, succinate). Surprisingly they all failed to rescue DRB18-induced neuronal insult. Interestingly, the cell-permeable TCA cycle intermediate analogue, dimethyl-2-oxoglutarate (DMO), significantly prevented DRB18-induced loss of cell viability, ROS production, mitochondrial membrane depolarization, and ATP depletion. These findings support the previous model of glucose hypometabolism in pathogenesis of AD. Moreover, it gives mechanistic insight that glucose hypometabolism mediated neuronal insult in AD is predominantly via mitochondrial bioenergetic failure. Our findings further validate that DMO holds promise as a neuroprotective agent worth exploring in the context of AD pathogenesis.

Disclosures: A. Ghosh: None. **A. Chauhan:** None. **A. Bir:** None. **S. Chakrabarti:** None. **S. Chakrabarti:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.14/E18

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

Support: MinCiencias Colombia proyecto 734

Title: Plant extract with potential liver X receptor (LXR) agonist activity as a therapeutic strategy against Alzheimer's disease.

Authors: *J. M. MUÑOZ[1], A. M. BUSTOS RANGEL[2], G. ARBOLEDA[3], A. G. SANDOVAL HERNANDEZ, Sr.[4];
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Abstract: Alzheimer's disease (AD) is characterized by the extracellular accumulation of β-amyloid (Aβ), the intracellular aggregation of hyperphosphorylated TAU protein, neuroinflammation, and calcium dyshomeostasis—key histopathological hallmarks that drive neurodegeneration. In the search for novel therapeutic strategies, the activation of type II nuclear receptors, such as liver X receptors (LXRs), has been explored. LXR activation has been shown to improve cognitive performance in the Morris Water Maze (MWM), reduce histopathological markers, and modulate biochemical parameters associated with synaptic function in the triple-transgenic mouse model of AD (3xTg-AD). This study evaluated the effect of the NR plant extract, an agonist of the liver X receptor (LXR), on cellular, molecular, transcriptomic, and behavioral alterations in experimental models of AD. Using in vitro approaches such as primary hippocampal neuron cultures and intracellular calcium imaging, along with in vivo assessments including the Morris Water Maze (MWM), and molecular biology techniques such as western blotting, immunofluorescence, and RNA sequencing (RNA-seq), we demonstrated that pretreatment with the NR plant extract confers neuroprotection against Aβ-induced toxicity in primary hippocampal neurons. Furthermore, chronic exposure to Aβ in these cultures led to a significant disruption of intracellular calcium homeostasis, as measured by calcium-sensitive fluorescent probes; this dysregulation was prevented by pretreatment with plant extracts possessing LXR agonist activity. In the 3xTg-AD mouse model, treatment with the ZM extract reduced Alzheimer's disease-related histopathological markers in the hippocampus and improved cognitive performance in the MWM task. Finally, RNA-seq analysis revealed that treatment with the NR extract induced differential expression of 3,744 transcripts, many of which are associated with pathways involved in glutamatergic synapse function and intracellular calcium regulation. The findings of this study support the therapeutic potential of LXR activation through bioactive plant-derived molecules as a multifaceted strategy to counteract the pathological processes associated with Alzheimer's disease. This approach not only promotes neuroprotective effects at the cellular and synaptic levels but also modulates key transcriptomic pathways involved in calcium homeostasis and glutamatergic neurotransmission, ultimately contributing to improved cognitive performance in experimental models of AD.

Disclosures: J.M. Muñoz: None. **A.M. Bustos Rangel:** None. **G. Arboleda:** None. **A.G. Sandoval Hernandez:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

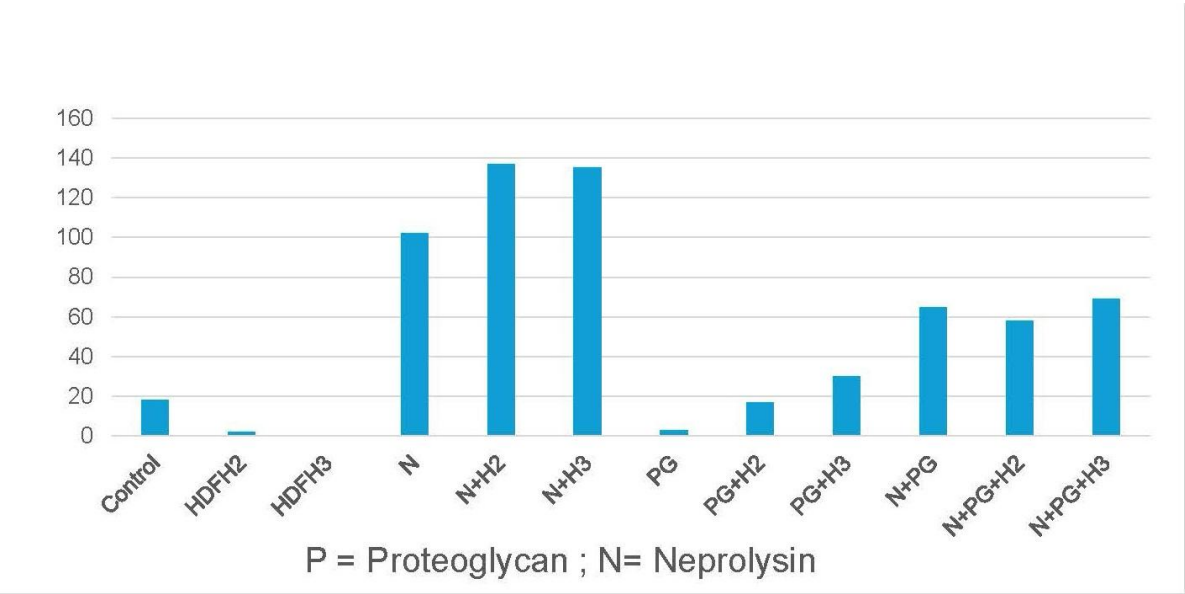
Program #/Poster #: PSTR066.15/E19

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

Title: Novel Compounds Potentiate the Action of Neprilysin in the Presence and Absence of Proteoglycan

Authors: *G. L. JONES, J. LEAVITT;
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Abstract: Given the desperate need to prevent Alzheimer's disease, we have undertaken the study of a small library (12 compounds of interest) of organic molecules which we have previously shown increase the activity of neprilysin and some other zinc-dependent enzymes. Our data is consistent with a primary action to alter the secondary structure of soluble amyloid beta 42 (AB42) oligomers thereby allowing for greater access for proteolysis. We have accepted the hypothesis that the soluble oligomers of AB42 are responsible for much of the pathology of Alzheimer's disease. We also recognize that certain proteoglycans inhibit the ability of these enzymes to metabolize amyloid. Thus, we undertook this study to determine whether proteoglycans might inhibit the ability of our compounds to increase such enzyme proteolysis of AB42 oligomers. We employed both electrophoresis and fluorescence spectroscopy in our efforts to better understand the interactions of two of our organic compounds (HDFH2 and HDFH3), neprilysin and proteoglycans with a range of AB42 oligomers. Please see the bar graph for further context. It is known that both HDFH2 and HDFH3 quench this amyloid fluorophore. The action of neprilysin is clearly appreciated in the fourth bar. However, rather than quench, both HDFH2 and HDFH3 increase the amplitude as can be seen. Proteoglycan alone either quenches the fluorescent signal or it does nothing to alter the secondary structure of amyloid, as inferred from the amplitude. The next two bars show that even in the presence of proteoglycan our two compounds both exert an effect to unquench the emission signal. We suggest this might be interpreted as altering the fold pattern of AB42 oligomers. Finally, in the last three bars, we see that even in the presence of proteoglycan, neprilysin still metabolizes amyloid although its effect is diminished and there appears to be no significant differential effect comparing HDFH2 and HDFH3. We hope that these data might increase awareness of molecular mechanisms that might govern the success of putative small molecule interventions.



Disclosures: G.L. Jones: None. **J. Leavitt:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

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Program #/Poster #: PSTR066.16/E20

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

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Italian Ministry of Health - Alternative Methods to Animal Testing Grant 2023 (NEURO-3R)

Title: Bezafibrate Rescues Tau-Induced Neurodevelopmental and Synaptic Deficits in a Human Cortical Organoid Model of MAPT-Linked Frontotemporal Dementia

Authors: *S. DI ANGELANTONIO[1], L. MAUTONE[2], C. D'ANTONI[2], F. CORDELLA[4], D. SALERNO[3], P. BEZZI[5];
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Abstract: Frontotemporal dementia (FTD) caused by mutations in the *MAPT* gene is characterized by early synaptic dysfunction, mitochondrial impairment, and progressive neuronal loss. The IVS10+16 intronic mutation alters MAPT pre-mRNA splicing, promoting exon 10 inclusion and leading to overproduction of 4-repeat (4R) tau isoforms—ultimately contributing to tau aggregation, hyperphosphorylation, and neurodegeneration. Despite this well-defined pathogenic mechanism, there are no disease-modifying therapies for MAPT-linked FTD, and early developmental alterations remain poorly understood. To model these processes, we generated 3D patterned cortical organoids from isogenic human induced pluripotent stem cells (iPSCs) harboring either the IVS10+16 *MAPT* mutation or wild-type control alleles. This organoid model enabled the study of both neurodevelopmental dynamics and later-stage neuronal function in a human context. We performed longitudinal molecular and functional assessments, including Nanostring gene expression profiling, immunofluorescence for synaptic and mitochondrial markers, and calcium imaging to assess network-level neuronal activity. Mutant organoids exhibited disrupted mitochondrial structure and bioenergetics, reduced synaptic marker expression, and impaired network activity, consistent with developmental tau pathology. Notably, bezafibrate—a pan-PPAR agonist known to stimulate mitochondrial biogenesis—restored mitochondrial content, normalized tau localization and phosphorylation, and rescued synaptic function and calcium dynamics in the mutant organoids. These results highlight the dual impact of the MAPT IVS10+16 mutation on early neuronal development and

synaptic integrity, and demonstrate that mitochondrial-targeted therapy can ameliorate both structural and functional deficits. Our study positions bezafibrate as a promising candidate for disease-modifying intervention in tauopathies and underscores the utility of cortical organoid models for bridging mechanistic insights with translational drug screening in human neurons.

Disclosures: **S. Di angelantonio:** F. Consulting Fees (e.g., advisory boards); D-Tails srl BC. **L. Mautone:** None. **C. D'Antoni:** None. **F. Cordella:** None. **D. Salerno:** None. **P. Bezzi:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

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

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Cure Alzheimer's Fund A069137

Title: Microplastic-induced Astrogliosis exacerbates Aβ-driven tau pathology in 3D human cellular models of Alzheimer’s disease

Authors: ***Y. RYOO**[1,2], J. PARK[1], S. KWAK[1], B. KANG[1], S. CHOI[1], J. LEE[2], R. E. TANZI[1], D. KIM[1];
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Abstract: Microplastics (MPs) are emerging environmental pollutants increasingly found in human tissues, including the brain. Recent studies have shown elevated MP accumulation in the brains of individuals with dementia, implicating a potential link to neurodegenerative diseases such as Alzheimer’s disease (AD) (Nihart et al., *Nature Medicine*, 2025). However, the mechanistic relationship between MP exposure and AD pathology remains unclear. Here, we investigated the impact of MPs on AD-associated neurodegeneration using a three-dimensional (3D) human neural cell culture model that recapitulates key features of AD, including amyloid-β (Aβ) plaque formation and Aβ-induced neurofibrillary tangle (NFT) pathology (Choi et al., *Nature*, 2014, Kwak et al., *Nat Commun*, 2020). To mimic the MP accumulation observed in human AD brains, we treated 3D AD cultures with polyethylene (PE) and polystyrene (PS) MPs at concentrations range from 0.2 to 5 μm. MP exposure did not induce overt cytotoxicity, as assessed by LDH release and fluorescence microscopy. Notably, MPs were robustly internalized by astrocytes, only in 3D AD cultures with elevated Aβ, not in control cultures. The AD astrocytes with endocytosed MPs exhibited high glial fibrillary acidic protein (GFAP) expression, enlarged somas, and marked upregulation of phospho-STAT3 and pro-inflammatory

cytokines, including IL-8, IFN-γ, and CCL2, all of which strongly indicate reactive astrogliosis. Importantly, MP exposure significantly enhanced Aβ-induced tau pathology in 3D AD cultures. Transcriptomic analysis further revealed that MP-induced upregulation of astrocyte activation genes was exclusively observed in AD cultures. To test whether the MP-induced astrogliosis plays a role in the elevated tau pathology, we generated astrocyte-depleted 3D AD cultures. Strikingly, removal of astrocytes completely blocked MP-induced GFAP expression and tau pathology, demonstrating that astrocytes are essential mediators of MP-driven AD pathology in our 3D AD cellular models. Together, our findings uncover a novel mechanism by which brain-accumulated MPs worsen AD pathology through astrocyte-dependent pathways. These results highlight the potential role of environmental pollutants in accelerating the progression of neurodegenerative diseases.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

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

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Title: Targeting The Microglia Receptor TREM2 In Alzheimer’s Disease: Structural and Biophysical Mapping of Functional Surfaces

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Abstract: The development of new innovative treatments to prevent and ameliorate Alzheimer’s disease (AD) requires knowledge of molecular mechanisms that are critical to neuronal health. The scavenger receptor TREM2 is part of a signaling complex that modulates inflammatory responses, phagocytosis and cell survival in microglia. In recent years, TREM2 has emerged as a promising drug target for AD. Understanding the molecular mechanisms underlying TREM2 signaling in microglia will facilitate the development of specific, safe and efficacious therapies for AD that target TREM2. Here we employed structural, biophysical, and functional studies to

identify the surfaces on TREM2 utilized to engage its various AD-associated ligands: amyloid beta; apoE; TDP-43; complement C1q; and IL-34. We developed a protein library of more than 25 different TREM2 variants that could be used to map interactions with ligands using biolayer interferometry (BLI). We used X-ray crystallography to determine the structure of a TREM2/amyloid beta peptide complex. We used cellular assays to investigate the functional impact of disrupting TREM2 interactions with ligands. We identified two major ligand-binding regions on TREM2: the hydrophobic site and the basic site. We found that mutations to the TREM2 hydrophobic site ablated binding to apoE, amyloid beta, and TDP-43. We found that mutations to the basic site ablated binding to IL-34, most dramatically by mutation of R76. We found that C1q binding was most strongly impacted by mutations to the basic site, including R46, with some mutations to the hydrophobic site also partially attenuating binding, suggesting a broader mediation of binding across the two sites. We determined the crystal structure of TREM2 in complex with an amyloid beta peptide, which confirmed binding to the hydrophobic site. Variants of amyloid beta which did not bind TREM2 were not effectively phagocytosed in a cellular assay, confirming functional significance of our findings. Our comprehensive analyses highlight the surfaces that TREM2 utilizes to engage ligands. The hydrophobic site appears to be a commonly used site for a number of ligands, so targeting this surface should mediate TREM2-ligand interactions involved in disease.

Disclosures: T. Brett: None. **J. Alexander-Brett:** None. **J. Greven:** None. **J. Wydra:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

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

Support: NIH/NINDS R01NS136448
K23NS109284
ROY J. CARVER Foundation
NIH/NINDS R01NS115800

Title: Tau Expression is Necessary for Maturation of Human Stem Cell-Derived Neurons

Authors: *B. DOLATYAR[1], N. FIROUZSHAHI[2], M. R. SHIN[3], D. GONZÁLEZ[5], J. GLYKYS[6], M. M. HEFTI[4];
[1]Univ. of Iowa, Iowa city, IA; [2]Neurosci., [3]Pathology, [4]Univ. of Iowa, Iowa City, IA; [5]Univ. Politécnica De La Zona Metropolitana D, Guadalajara, Mexico; [6]Pediatrics and Neurol., The Univ. of Iowa, Iowa City, IA

Abstract: Introduction: The tau protein is a major driver of neurotoxicity in the adult and aging brain. It is however, strongly evolutionarily conserved. This suggests that it plays a vital, but thus far unclear role earlier in life. Studies in rodent models have provided conflicting data and are

difficult to interpret due to differences in tau amino acid sequence, while human studies have focused mostly on tau's role in neurodegeneration. This study aims to provide the first comprehensive analysis of developmental phenotypes in human tau knockout neurons. **Methods:** Human embryonic stem cells (hESCs) were used to generate mixed dorsal forebrain cultures, equivalent to human second-trimester fetal neurons. We used western blotting, quantitative PCR, immunocytochemistry, electrophysiology, lentiviral transfection, and RNA sequencing to characterize cellular phenotypes. Image quantification was done using MATLAB and/or ImageJ. **Results:** Tau knockout neurons after fourteen days of differentiation show decreased staining for TUJ1 (p=.004), which marks all neuronal processes, but not MAP2, which specifically marks soma and dendrites. This suggests that the primary processes involved are axons rather than dendrites. We then sought to assess synaptic integrity and found overall decreased staining and increased localization to the soma for PSD-95 (p=0.007), with similar findings for synaptophysin. There was no decrease in overall levels of PSD-95 or synaptophysin. We then showed that both TUJ1 and PSD-95 deficits could be rescued by transfection with 0N3R-RFP tau. We also saw broader deficits in maturation, including fewer total neurons (p=0.0395), higher input resistance (386 MΩ vs. 1154 MΩ, p=0.0053), and longer time constant (34.6 vs. 62.96 ms, p=0.0335) in tau knockout neurons. RNA sequencing comparing wildtype and tau knockout neurons showed downregulation of 698 genes and upregulation of 1169 in response to tau knockout. Downregulated genes were enriched for gene ontology terms related to neuron differentiation. **Discussion:** The results of this study suggest that tau plays a crucial role in the maturation of human neurons, whereas this may not be the case in mouse neurons. These findings suggest a previously unknown role for tau in human synaptogenesis and neuronal maturation, as well as providing valuable insights into processes relevant to Alzheimer's and other tauopathies. The mechanism by which tau influences transcription remains unclear, opening possibilities for future research in this area.

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Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.20/E24

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

Support: RF1-AG077772
R01-AI173804
Functional Multi-omics of Aging (FMATG) training grant

Title: Evaluating the Disruption of the Mitochondrial Network in an in vitro model of Alzheimer's Disease

Authors: *K. PERSON[1], W. C. LOW[2];
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia in adults 65 years or older. Despite its widespread impact on a sizeable portion of the population, the treatment options are limited. AD is associated with mitochondrial ultrastructure changes, and significant alterations in proteins that regulate mitochondrial fission and fusion, and oxidative stress. Thus, mitochondrial dysfunction is a key mediator of AD pathophysiology. Initial reports indicated that transfer of healthy mitochondria enhanced recipient cell viability by increasing ATP production and protecting cells from metabolic damage.In the CNS mitochondria are released from several cell types including astrocytes, microglia, and cerebrovascular endothelial cells. These mitochondria function as non-cell autonomous communicators mediating cell to cell cross talk with tremendous potential to protect or repair damaged cells. We postulated that the engulfment of pathogenic proteins such as amyloid beta 42 (Aβ42) and hyperphosphorylated tau by microglia results in the interaction of these proteins with mitochondria that disrupts mitochondrial function, and in turn, leading to chronic mitochondrial dysfunction. To test this, we conducted in vitro studies to assess the mitochondrial network following Aβ1-42 in BV2 microglial cells in tissue culture. Using this model, we will present data on the functional and morphological alterations of the mitochondrial network following Aβ1-42 toxicity in microglial cells.

Disclosures: K. Person: None. **W.C. Low:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.21/E25

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

Title: Ultrasensitive, High-Throughput Multilayer Cerebral Organoid Sensor for Accelerated Alzheimer's Therapeutics Development

Authors: *A. N. BEGUM[1], N. N. BHATT[2], R. KAYED[2], A. V. MICHKOV[1];
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Abstract: Alzheimer's disease affects 7.2 million people in the U.S. aged 65 and older in 2025, with annual healthcare costs reaching $781 billion. Traditional preclinical drug testing relies on cellular and animal models; however, species differences contribute to failure rates exceeding 85 percent. To address these limitations, NeyroblastGX LLC developed ADBrainSense™, a high-throughput cerebral organoid system integrating electroconductive sensing arrays for rapid and cost-effective drug screening. NeyroblastGX's patent-pending OrGspheroid™, derived from human-induced pluripotent stem cells, ensures consistent size and cytoarchitecture in cerebral

organoids. To enhance physiological relevance, cortical neuron- and astrocyte-enriched cerebral organoids were co-cultured with microglia and microvascular endothelial progenitor cells, replicating the complexity of the central nervous system. Immunocytochemistry confirmed key markers, including neuronal beta tubulin III, TBR1, TBR2, SATB2, FOXP2, astrocytic GFAP, microglial IBA1, endothelial microvascular VE-cadherin, and excitatory synaptic vGlut1, PSD95, and synaptophysin. Additionally, cerebral organoids exhibited greater than 80 percent cellular viability after freeze-thaw cycles, assessed via MTT assay, demonstrating high reproducibility, minimal batch-to-batch variation, and no necrosis formation over 90 days. Cerebral organoids were optimized for growth on high-throughput sensor plates equipped with Electric Cell-Substrate Impedance Sensing electrodes in a 96-well format, allowing real-time electrophysiological current responses to amyloid-beta and tau oligomers at concentrations of 10 and 50 micrograms. Notably, the combined treatment of amyloid-beta and tau oligomers resulted in significant differences in electric current at a frequency of 64,000 hertz, providing insights into neurodegenerative activity. Immunocytochemistry analysis revealed synaptic marker loss, specifically PSD-95 and synaptophysin, following exposure to 50 micrograms of combined amyloid-beta and tau oligomers, correlating with electrophysiological sensor signals. This high-throughput platform enables the identification of Alzheimer's disease-related electrophysiological signals within three hours using 96 mini-organoids, whereas traditional rodent models may require approximately 100 mice over six months, making them time-consuming and costly. Our system provides a fast, scalable, and cost-effective approach for drug screening, significantly accelerating the discovery of Alzheimer's disease therapeutics in a physiologically relevant human model.

Disclosures: **A.N. Begum:** A. Employment/Salary (full or part-time): NeyroblastGX LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeyroblastGX LLC. **N.N. Bhatt:** None. **R. Kayed:** None. **A.V. Michkov:** A. Employment/Salary (full or part-time): NeyroblastGX LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeyroblastGX LLC.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.22/E26

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

Support: BrightFocus Foundation

Title: The role of pathological protein expression in Sox10-lineage cells in neurodegeneration

Authors: F. MAYRHOFER[1], V. PATEL[2], C. SADEGH[3], ***O. CHECHNEVA**[1];
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Abstract: Aggregation and spreading of pathological proteins in neurons underlie many genetic and age-related neurodegenerative disorders affecting memory, cognition, and movement, including frontotemporal dementia (FTD), Alzheimer disease and amyotrophic lateral sclerosis (ALS). However, the mechanisms by which pathological proteins accumulate and spread remain poorly understood, limiting the development of effective therapies. Until recently, research on pathogenic protein accumulation has focused primarily on neurons. We found that in the healthy mouse central nervous system, neurons accumulate nuclear and ribosomal material derived from Sox10-lineage cells (SOL), including oligodendroglia. While the biological role of this process remains unclear, we hypothesize that it may contribute to the spreading of pathological proteins in neurons in neurodegenerative disorders. TAR DNA-binding protein 43 (TDP-43) is a nuclear RNA-binding protein involved in RNA metabolism. Neuronal TDP-43 aggregates are hallmark features of FTD and ALS. We generated transgenic mice expressing a mutant human form of TDP-43 in SOL. Using Cre-dependent fluorescent reporter mKate, we confirmed that Cre-induced recombination was specific to SOL. After induction of hTDP-43 expression, we examined behavioral phenotype and brain pathology. Together, our findings suggest that SOL may play a critical role in the intercellular transfer of pathological TDP-43 to neurons, supporting the hypothesis that glia-derived pathological proteins contribute to neuronal dysfunction and degeneration.

Disclosures: F. Mayrhofer: None. **V. Patel:** None. **C. Sadegh:** None. **O. Chechneva:** None.

Poster

PSTR066: Alzheimer's Disease Therapeutic Strategies: *In Vitro* Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR066.23/E27

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

Support: Alzheimer’s Association AARF-22-974258
Florida Department of Health 22A07
National Institute of Aging (NIA) R56 AG062556

Title: Knock out of UFSP2 to model disease-associated hyperUFMylation in cells

Authors: *T. YAN[1], M. HECKMAN[2], M. E. MURRAY[3], D. W. DICKSON[4], W. SPRINGER[5], F. C. FIESEL[2];
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Abstract: Background: UFM1 is a small ubiquitin-like modifier that has been implicated in neurodevelopmental disorders. Strikingly, the UFM1 cascade has recently been identified as a major modifier of tau aggregation in cells and this was confirmed in animal models with pathological tau. Furthermore, our group recently reported profound changes in the frontal and temporal cortex of Alzheimer’s disease (AD) brain compared to neurologically unaffected

controls. These changes included the significant increase of conjugated UFM1 and the reduction of soluble UFSP2. In line with the known role of UFSP2 as the UFM1-deconjugation enzyme. both molecular changes were highly negatively correlated with each other. **Methods:** We have used CRISPR-Cas9 mediated knock out UFSP2 in order to study the loss of UFSP2 function in cells. In neurons, upon induction of either DNA damage or ER stress, both of which are prominent features of AD, we assessed the stress response compared to non-edited WT cells and used UFM1 KO cells as controls using imaging, biochemistry and molecular readouts. In HEK293 UFSP2 KO cells we further investigated modification of tau itself using denatured immunoprecipitation and mass spectrometry. **Results:** In all investigated cell types, the loss of UFSP2 caused a significant increase in conjugated UFM1. In neurons, and upon DNA damage, this led to increased survival in a UFM1-dependent manner. Upon ER stress, we found that both hyperUFMylation and the loss of UFM1 caused increased ER stress response. In HEK293 cells, the loss of UFSP2 leads to marked increase of conjugated UFM1 as well. In these cells, upon overexpression of UFM1 conjugation proteins, we found that UFM1 was conjugated to tau itself and confirmed this by mass spectrometry. **Conclusions:** Our findings demonstrate that loss of the UFM1-specific protease UFSP2 causes a significant increase in conjugated UFM1, linking UFMylation to stress responses and tau pathology in AD. This hyperUFMylation enhances neuronal survival under DNA damage but worsens ER stress. Identifying tau as a direct UFM1 substrate suggests that modulating the UFM1-UFSP2 axis could offer new therapeutic strategies for AD and related tauopathies.

Disclosures: T. Yan: None. **M. Heckman:** None. **M.E. Murray:** None. **D.W. Dickson:** None. **W. Springer:** None. **F.C. Fiesel:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.01/E28

Topic: C.03. Parkinson’s Disease

Support: MJFF-022679
U19-NS110456

Title: In vitro characterization of PARP-1 expression in mouse models of α-synucleinopathies and post-mortem human Lewy body dementia brain tissue.

Authors: *S. SAKEER[1], Y. ZHU[2], D. SATURNINO GUARINO[1], A. YOUNG[1], E. B. LEE[3], K. C. LUK[3], R. H. MACH[1];
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Abstract: PARP-1, a poly (ADP-ribose) polymerase, is predominantly triggered by DNA damage. Overexpression of PARP-1 is associated with oxidative stress, neuroinflammation and mitochondrial dysfunction, contributing to the pathogenesis of several chronic neurological disorders (CNDs), including Lewy body dementias (LBDs). PARP-1 has been found to generate PAR units which attach to alpha synuclein (αSyn) and PARylated αSyn is more aggregable, leading to a feed-forward loop of toxicity. Thus, PARP-1 inhibition could offer therapeutic opportunities for managing CNDs. The goal of this study was to evaluate PARP-1 expression as an emerging biomarker in the progression of α-synucleinopathies using murine models of PD and post-mortem human brain tissue from patients with LBDs. To achieve this, we applied in vitro autoradiography (ARG) with tritium labeled FTT, an analog of rucaparib (FDA-approved PARPi) and immunofluorescence (IF) staining of PARP-1, αSyn and microglia. In details, the mouse models used were: transgenic αSyn mouse model (line M83) injected with αSyn preformed fibrils (PFFs), M83 mice injected with human healthy control (HC), multiple system atrophy (MSA) and LBD brain lysate and the Thy1:*SNCA/Snca*$^{-/-}$ mouse model injected with human amplified Lewy bodies (sacrificed 3, 6, 9 months post-injection [MPI]). Very low signal with [^{3}H]FTT (10nM) was detected in PFF-injected M83 mice indicating a low PARP-1 expression in this murine model. The binding profile of [^{3}H]FTT in M83 injected with human brain lysates was the following: MSA>LBD>HC, suggesting a higher expression of PARP-1 in the presence of MSA derived a-synuclein deposits. [^{3}H]FTT binding in Thy1:*SNCA/Snca*$^{-/-}$ mice sacrificed at 3, 6 and 9MPI had the highest intensities at 6MPI; ARG tracer binding and PARP-1 and α-syn IF correlated significantly in the hippocampus (R^2=0.8, p=0.05) of this model. [^{3}H]FTT specific binding in human post-mortem brain samples was higher in PD, PDD and DLB brains compared to healthy, age-matched control cases. Our data implicates an overactivation of PARP-1 in a few murine models of PD and in LBDs human brain tissue, supporting the rationale of considering PARP-1 an emerging therapeutic target in neurodegeneration.

Disclosures: S. Sakeer: None. **Y. Zhu:** None. **D. Saturnino Guarino:** None. **A. Young:** None. **E.B. Lee:** None. **K.C. Luk:** 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.; MJFF. **R.H. Mach:** 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.; MJFF, NIH.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.02/E29

Topic: C.03. Parkinson’s Disease

Title: Translational EEG Rat Model of Dyskinesia for Dopaminergic Drug Evaluation in Parkinson's Disease

Authors: N. VAUTRELLE[1], *V. DUVEAU[1], A. EVRARD[1], R. RODRIGUEZ SARMIENTO[2], E. C. O'CONNOR[2];
[1]Synapcell SAS, St Ismier, France; [2]Pharma Res. and Early Develop., Roche Innovation Ctr., Basel, Switzerland

Abstract: ***Background:*** Parkinson's disease (PD) is a neurodegenerative disorder marked by motor symptoms such as tremor, rigidity, and bradykinesia, and linked to increased beta-band oscillatory activity in the brain. While L-DOPA is the standard-of-care for symptomatic relief in PD, its chronic use often leads to motor complications, most notably Levodopa-Induced Dyskinesias (LID), a debilitating condition correlated with gamma-band oscillations and driven by fluctuating plasma drug levels due to L-DOPA's short half-life. To improve the quality of life for PD patients, there is a pressing need for novel treatments with greater efficacy, longer duration, and fewer side effects. ***Objective:*** We aimed to establish a translational rat model of LID, combined with EEG recordings, to simultaneously assess both efficacy and dyskinesia liability. We assessed the predictive nature of the model by testing clinically validated dopaminergic replacement therapies, including L-DOPA and the non-catecholamine D1/D5 receptor agonist Tavapadon, which has demonstrated efficacy in Phase 3 studies for PD. ***Methods:*** Dopaminergic lesions were induced in Sprague-Dawley rats via intracerebral 6-hydroxydopamine injections, and rats were implanted with cortical electrodes for resting-state EEG recordings. Dyskinesias were induced by daily intraperitoneal injections of L-DOPA (6 mg/kg) and benserazide (12 mg/kg) for 15 days. ***Results:*** The model replicates key human biomarkers: (1) pharmaco-sensitive beta oscillations for evaluating anti-parkinsonian efficacy, and (2) gamma oscillations and abnormal involuntary movements (AIMs) post-LID induction for quantifying dyskinesia. Tavapadon showed prolonged beta suppression compared to L-DOPA, likely reflecting its superior pharmacokinetic properties. Tavapadon also showed a superior side effect profile, inducing fewer AIMs and lower gamma power than L-DOPA at particular dose levels. ***Conclusions:*** These findings demonstrate that combining EEG biomarkers with behavioral scoring in our rat model of LID offers a robust platform to evaluate the therapeutic profiles of novel dopaminergic agents. This model is well suited for preclinical screening of compounds with the potential to provide sustained motor benefits while minimizing treatment-emergent dyskinesias, or to de-risk candidates with possible pro-dyskinetic effects before clinical development. Finally, our findings with Tavapadon align with a Parkinsonian non-human primate study (Young et al., ACS Chem. Neurosci. 2020 Feb 19; 11(4): 560-566), suggesting the rat model's suitability as an alternative to monkeys in evaluating novel dopaminergic treatments for PD.

Disclosures: N. Vautrelle: A. Employment/Salary (full or part-time): Synapcell SAS. **V. Duveau:** A. Employment/Salary (full or part-time): Synapcell SAS. **A. Evrard:** A. Employment/Salary (full or part-time): Synapcell SAS. **R. Rodriguez Sarmiento:** A. Employment/Salary (full or part-time): Hoffmann-La Roche Ltd. **E.C. O'Connor:** A. Employment/Salary (full or part-time): Hoffmann-La Roche Ltd.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.03/E30

Topic: C.03. Parkinson's Disease

Support: WBI TR_2017

Title: Effect of the combination of trehalose and trehalase inhibitors in a rat model of Parkinson's disease

Authors: *P. A. HOWSON[1], J. M. BROTCHIE[2], M. P. HILL[2], T. H. JOHNSTON[2];
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Abstract: Trehalose is being developed as a potential oral therapeutic for Parkinson's disease (PD). Following oral ingestion of trehalose, the majority is metabolised to glucose by trehalase in the wall of the jejunum and duodenum, thus large, daily amounts of oral trehalose are required to produce beneficial effects in animal models of PD. We hypothesised that trehalase inhibition in the gastrointestinal tract would allow a greater proportion of orally administered trehalose to reach systemic circulation and thus would allow the daily dose of trehalose to be reduced whilst still maintaining the beneficial effects.
To investigate this we co-administered a known efficacious dose of trehalose (2.67 g/kg) and two trehalase inhibitors, tannic acid (50 and 150 mg/kg) and validamycin A (0.175 and 0.525 mg/kg) and demonstrated that trehalase inhibition increased both plasma and brain trehalose exposure. We next examined how much we could reduce the dose of trehalose, in the presence of trehalase inhibitors, whilst still maintaining trehalose exposure and demonstrated that we could reduce the daily dose of trehalose by up to 75%.
Finally, we investigated whether the beneficial effect of high-dose trehalose alone could be maintained when a low-dose of trehalose was co-administered with trehalase inhibitors in an AAV1/2-hA53T-aSyn rat model of PD. As expected, the rat model presented with significant reductions in striatal dopamine (51%), striatal DAT (20%), and the number of TH^{+ve} cells in the substantia nigra (39%) and the combination of low-dose trehalose and trehalase inhibitors significantly improved these parameters back to 75% (dopamine), 90% (DAT), and 94% (TH) of control levels.
In conclusion, we demonstrated that trehalase inhibition allows the daily dose of trehalose to be reduced whilst preserving systemic trehalose exposure and neuroprotective effects in a rat model of PD.

Disclosures: P.A. Howson: A. Employment/Salary (full or part-time): Atuka Inc.,. **J.M. Brotchie:** A. Employment/Salary (full or part-time): Atuka. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka. **M.P. Hill:** A. Employment/Salary (full or part-time): Atuka. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka. **T.H. Johnston:** A. Employment/Salary (full or part-

time): Atuka. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Atuka.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.04/E31

Topic: C.03. Parkinson's Disease

Support: Midwestern University
Ben and Catherine Ivy Foundation

Title: Assessing motor behavior and dopamine neuron mitochondrial redox status in parkin-null Drosophila treated with a redox-active manganese porphyrin compound

Authors: A. N. FOLEY[1], A. N. JUBA[2], T. MARGARYAN[3], T. JONES[1], I. BATINIC-HABERLE[4], A. TOVMASYAN[5], *L. M. BUHLMAN[1];
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Abstract: Autosomal-recessive-Juvenile Parkinsonism (AR-JP) is a hereditary neurodegenerative disorder with typical onset before age 40 that is characterized by degeneration of dopaminergic cells in the substantia nigra. The *parkin* gene is conserved in *Drosophila* and mammals; loss-of-function *parkin* mutations cause AR-JP in humans and climbing deficits in flies that are associated with dopaminergic neurodegeneration. Oxidative stress is heavily implicated in dopaminergic neurodegeneration in all forms of Parkinson's disease (PD) and in parkin-null *Drosophila*. We previously demonstrated that parkin-null *Drosophila* dopaminergic neuron mitochondria have glutathione redox disequilibrium. MnTE-2-PyP^{5+} is a redox-active manganese porphyrin (MnP) that has been extensively studied in both preclinical and clinical settings for disease conditions involving oxidative stress. To determine whether MnTE-2-PyP^{5+} can improve the parkin-null *Drosophila* phenotype, we continuously administered 10 μM MnTE-2-PyP^{5+} in standard *Drosophila* food and evaluated survival, as well as climbing motivation and ability. While whole brain concentration, as determined by LC-MS/MS, was similar between genotypes and sexes, the MnTE-2-PyP^{5+} improves survival in control but not parkin-null flies and did not affect climbing behavior. Using redox-sensitive fluorescent reporters, we further determined that MnTE-2-PyP^{5+} did not restore disrupted glutathione redox equilibrium in dopaminergic neuron mitochondria. Interestingly, our team has observed that a next generation MnP (with likely differential bioavailability across brain regions and organelles) enhances climbing behavior and glutathione redox equilibrium in female parkin-null flies. These results warrant further investigation of newer-generation MnPs that possess similar redox activity while offering superior bioavailability.

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Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.05/E32

Topic: C.03. Parkinson's Disease

Support: RS-2025-02213938

Title: Evaluating the role of novel ASK1 inhibitors in preclinical models of Parkinson's disease

Authors: *S.-Y. NA[1], B. KO[1], J. KIM[1], Y. KIM[1], S. LEE[1], J. LEE[1], K. PARK[1], P. J. SWEENEY[1], J. SONG[2], K. LEE[2], L. PARK[1];
[1]Naason Sci. Inc., Cheongju-si, Chungbuk, Korea, Republic of; [2]KRICT, Deajeon, Korea, Republic of

Abstract: Apoptosis signal-regulating kinase 1 (ASK1) is a redox-sensitive MAP3K that activates downstream JNK and p38 MAPK pathways, contributing to neuronal apoptosis and neuroinflammation in neurodegenerative diseases. Here, we report the preclinical evaluation of two novel ASK1 inhibitors, structurally derived from senolsertib, in both cellular and animal models of Parkinson's disease (PD). In vitro, both compounds effectively suppressed LPS-induced inflammatory responses in BV-2 microglial cells, significantly reducing mRNA expression of TNF-α, IL-1β, and IL-6, as well as TNF-α protein production. In primary astrocytes, the inhibitors improved cell viability under oxidative stress induced by H_2O_2, indicating a protective effect against ROS-mediated cytotoxicity. In vivo efficacy was evaluated in two murine PD models: the MPTP-induced model and the mThy1 α-synuclein transgenic mouse model. In the MPTP model, mice treated with the ASK1 inhibitors exhibited improved motor performance, as evidenced by reduced turn times and climb-down times in the pole test. This improvement was greater than that observed with nilotinib. Immunohistochemistry from ASK1 treated MPTP mice revealed increased tyrosine hydroxylase (TH) expression in both the striatum and substantia nigra, along with elevated dopamine levels in the substantia nigra. In the α-synuclein transgenic model, ASK1 inhibition significantly reduced step errors in the challenge beam test, overall increased TH expression in the striatum, and decreased Iba1-positive microglial activation in the cortex. Collectively, our findings demonstrate that ASK1 inhibition confers robust anti-inflammatory and neuroprotective effects in both early and progressive models of PD. These results support further development of ASK1-targeted therapeutics as potential disease-modifying treatments for Parkinson's disease.

Disclosures: S. Na: None. **B. Ko:** None. **J. Kim:** None. **Y. Kim:** None. **S. Lee:** None. **J. Lee:** None. **K. Park:** None. **P.J. Sweeney:** None. **J. Song:** None. **K. Lee:** None. **L. Park:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.06/E33

Topic: C.03. Parkinson’s Disease

Title: Probing the phosphorylation state of ABL1 (c-Abl) kinase and one of its targets, SNCA (alpha-synuclein), using ABL inhibitors.

Authors: *G. A. O'SULLIVAN;
Astex Pharmaceuticals, Cambridge, United Kingdom

Abstract: Gregory A. O’Sullivan on behalf of Astex.
Oxidative stress and mitochondrial dysfunction play a role in dopaminergic neuron degeneration in Parkinson’s Disease (PD). The tyrosine-protein kinase ABL1 is activated by oxidative stress and has been shown to phosphorylate PD proteins such as SNCA (alpha-synuclein), PRKN (Parkin) and ZNF746 (PARIS), targets implicated in PD. Further, published pre-clinical *in vivo* data, involving several different ABL inhibitors, has shown that this pathogenic stress response can be dampened and that downstream targets such as SNCA aggregates can be reduced. As a result, several different ABL inhibitors have been and continue to be evaluated in the clinic for synucleinopathies such as PD. In our efforts to develop novel proprietary ABL1 inhibitors, we have generated robust and quantitative assays to measure stress induced autophosphorylation of ABL1 and one of its reported substrates, SNCA. Whilst previous published work had shown that ABL1 activation can be assessed by its phosphorylation state at tyrosine Y245 or Y412, the low endogenous expression level of ABL1 in mouse brain and sub-optimal sensitivity of the commonly used assays limited their use as brain biomarkers. Instead, we show that phosphorylation of another tyrosine residue (Y204) is also induced in response to oxidative stress and PDGFR signalling *in vitro* and reduced by treatment with ABL inhibitors both *in vitro* and *in vivo*. Further, a custom-made ultra-sensitive SIMOA assay successfully detected phosphorylation of Y204 in mouse brain where endogenous levels of ABL1 are known to be low. We also developed an assay to measure phosphorylated Y39 SNCA in HEK293 cells through the overexpression of both ABL1 and SNCA. Treatment of these cells with known orthosteric or allosteric ABL1 inhibitors reduced the number of phosphorylated Y39 SNCA positive aggregates. In conclusion, we have developed (1) cell-based assays to quantify ABL inhibitor mediated phosphorylation of ABL1 at Y204 and SNCA at Y39 and (2) an ultrasensitive assay to quantify activated/phosphorylated ABL1 in rodent brain. These results and assays should prove useful in the further evaluation of Astex proprietary ABL inhibitors that have been designed using our fragment and structure-based drug discovery approaches. "Astex is committed to the ethical use of animals and adheres to the principles of 3Rs (see www.astx.com)"

Disclosures: G.A. O'Sullivan: A. Employment/Salary (full or part-time): Astex Pharmaceuticals, Cambridge, UK.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.07/E34

Topic: C.03. Parkinson's Disease

Title: Csm-101, a small molecule agonist of trpml1 for parkinson's-related disorders

Authors: *L. O. MURPHY, J. GARCIA FORTANET, D. BAIRD, T. LEHMBERG, K. SKERRY, M. BROADUS;
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Abstract: OBJECTIVE (i) To develop a brain-penetrant small molecule agonist for the lysosomal TRPML1 channel and, (ii) To perform preclinical studies supporting developing in Parkinson's-related disorders. BACKGROUND Lysosomal homeostasis is critical for efficient metabolism of certain proteins and lipids which would otherwise accumulate leading to cellular stress and pathologies. Alterations in the endolysosomal pathway are common in Parkinson's disease (PD) and related disorders such as Gaucher's disease (GD). Heterozygous loss of function mutations in *GBA1*, which encodes the lysosomal enzyme GCase, are reported in 15-20% of PD and increase disease severity and age of onset. Hypomorphic *GBA1* mutations are common and fully penetrant in GD. We developed CSM-101 as a novel small molecule agonist for the lysosomal ion channel TRPML1 and a drug candidate for PD and GD. METHODS Cellular pharmacological profiling of CSM-101 was performed using assays including whole-cell electrophysiology and high content imaging. CSM-101 was used to treat primary mouse cortical neurons incubated with conduritol beta epoxide (CBE), a GCase inhibitor. Specific cellular lipids levels were determined using LC-MS. Mice were treated with CBE (50 and 100 mg/kg delivered ip) and effects of CSM-101 on survival were determined. Rats with unilateral overexpression of α-synuclein (hA53T) in substantia nigra were treated with CSM-101 for 6 weeks. RESULTS CSM-101 is a selective and potent TRPML1 agonist that is well tolerated in rodents. In the primary neuron CBE model, CSM-101 significantly reduced levels of glucosylsphingosine. Importantly, the premature lethality seen in CBE-treated mice was significantly delayed following daily dosing with CSM-101. This effect was accompanied by a significant lowering in circulating levels of NfL. *Gba1*$^{D409V/D409V}$ knock-in mice, a genetic model for GD, have elevated brain glucosylceramides and after treatment with CSM-101 these lipids were significantly reduced. Moreover, neuroinflammation in *Gba1*$^{D409V/D409V}$ mice was normalized by CSM-101. Finally, CSM-101 reduced levels of α-synuclein by 30% and preserved tyrosine hydroxylase neurons in rats. CONCLUSIONS CSM-101 is a promising drug candidate associated with significant efficacy in preclinical models of GD and PD. The findings support

the concept that targeting lysosomal homeostasis via activation of TRPML1 is an attractive therapeutic approach.

Disclosures: L.O. Murphy: None. **J. Garcia Fortanet:** None. **D. Baird:** None. **T. Lehmberg:** None. **K. Skerry:** None. **M. Broadus:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.08/E35

Topic: C.03. Parkinson's Disease

Support: MJFF

Title: Novel small molecules targeting NURR1 are associated with robust neuroprotection in an AAV1/2 human A53T alpha-synuclein rat model of Parkinson's disease

Authors: T. MAGNUS[1], *M. P. HILL[2], C. SKEITH[1], T. H. JOHNSTON[1], P. A. HOWSON[1], P. NOYMER[3], P. M. ENGLAND[4,3];
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Abstract: NURR1 is critical for the development, growth, maintenance and survival of midbrain dopaminergic neurons. NURR1 regulates expression of all genes required for dopamine synthesis, packaging, and reuptake and in PD patients NURR1 expression levels are reduced. NURR1 also up-regulates anti-oxidative stress genes in neurons and down-regulates expression of proinflammatory genes in glial. The aim of this study was to evaluate, in a rat model of nigrostriatal synucleinopathy, the neuroprotective potential of two novel small molecules that act by stabilizing the NURR1 protein in two different conformations, one that regulates neuroinflammation (TI 1) and the other that regulates dopamine homeostasis and oxidative stress (TI 2). On Day 1 (D1), groups of adult female SD rats (Charles River) received stereotaxic unilateral nigral injection of AAV1/2-hA53T-aSyn (Groups 2-6) or empty-vector control (Group 1). From D2 through D42, animals were administered vehicle (Groups 1 and 2), Test Item 1 (TI 1) at 32 mg/kg (Group 3) or 16 mg/kg (Group 4) or Test Item 2 (TI 2) at 80 mg/kg (Group 5) or 40 mg/kg (Group 6). Forelimb asymmetry was assessed via the cylinder test at baseline, D22 and D42. Dopaminergic endpoints included striatal dopamine levels (assessed via LC-MS/MS), dopamine transporter (DAT) binding (via autoradiography), and nigral TH-positive (TH^+), α-synuclein (aSyn - HA tag)-positive, and NeuN-positive cell counts (via triple-stain IF and stereology). Microglial and astrocytic reactivity were evaluated via Iba-1 and GFAP IHC, respectively. Total and aggregated aSyn levels in the striatum were measured via ELISA. TI 1 and T1 2 administration were both associated with a significant reduction in forelimb asymmetry compared to vehicle treatment and a reversal of aSyn-induced striatal dopamine loss (by 91% -

Group 3; 88% - Group 5; 69% - Group 6). Follow-up studies with tissue from animals administered T1 2 show nigral TH^+ cell counts were increased by 61% and TH^+ cells expressing the aSyn transgene were significantly reduced (by 21%) compared to vehicle (Group 3). Markers of neuroinflammation revealed significantly decreased microglial and astrocytic reactivity in the SN of animals from Group 3. Treatment with a NURR1 ligand produced a robust neuroprotective effect positively modulating both dopaminergic and neuroinflammatory endpoints with associated functional (behavioural) benefit. These results suggest that modulation of NURR1 may represent a promising strategy for disease-modifying therapy in Parkinson's disease.

Disclosures: T. Magnus: None. **M.P. Hill:** None. **C. Skeith:** None. **T.H. Johnston:** None. **P.A. Howson:** None. **P. Noymer:** None. **P.M. England:** A. Employment/Salary (full or part-time): UCSF.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.10/E36

Topic: C.03. Parkinson's Disease

Support: CSIR 09/1252(0001)/2019
Higher Education Department, Government of Kerala, IN

Title: Nanoformulated asiatic acid-enriched Centella asiatica confers neuroprotection by targeting oxidative stress and mitochondrial dysfunction in Parkinson's disease

Authors: *C. V. SAJI, R. A. SHENOI, G. CHANDRA, K. P. MOHANAKUMAR;
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Abstract: Parkinson's disease (PD), involves progressive dopaminergic neurodegeneration, linked to oxidative stress, mitochondrial dysfunction, and reduced expression of tyrosine hydroxylase (TH) and dopamine transporter (DAT). This exploratory study evaluated the neuroprotective effect of asiatic acid (AA), *Centella asiatica* extract enriched in AA (Ext) and their poly (lactic-co-glycolic acid) based nanoformulations (*nf*-AA and *nf*-Ext) in SH-SY5Y neurons treated with 1-methyl-4-phenylpyridinium (MPP^+) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced PD mice. Cell viability (MTT assay), reactive oxygen species levels (DCFH-DA), mitochondrial membrane potential (JC-1), and mitochondrial super oxide levels (MitoSox red) were assessed in triplicates across independent experiments. TH and DAT expressions were quantified by immunoblotting. *In vivo*, MPTP-induced motor deficits (pole, rotarod, and swim tests) and striatal dopamine levels (HPLC-ECD) were evaluated after pretreatment with *nf*-AA and *nf*-Ext. In SH-SY5Y neurons, MPP^+ reduced viability, increased ROS, depolarized mitochondria, and decreased TH/DAT expression. Pretreatment with Ext and

AA partially reversed these effects, whereas *nf*-AA and *nf*-Ext significantly attenuated ROS generation, preserved mitochondrial membrane potential, and restored TH/DAT expression. In MPTP-induced PD mice, *nf*-Ext and *nf*-AA markedly mitigated dopamine loss and significantly improved motor functions in the behavioural tests, outperforming their unformulated counterparts. These behavioural improvements aligned with restoration of striatal dopamine loss, support the functional efficacy of nanoformulations in reversing PD like deficits. These findings demonstrate that nanoformulated Ext and AA enhance cellular uptake and bioavailability, providing superior neuroprotection via antioxidant and mitochondrial stabilization mechanisms. This supports the therapeutic potential of nano herbal formulations in PD and highlights their translational value for overcoming pharmacokinetic limitations of phytochemicals.

Disclosures: C.V. Saji: None. **R.A. Shenoi:** None. **G. Chandra:** None. **K.P. Mohanakumar:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.11/E37

Topic: C.03. Parkinson’s Disease

Support: Indian council of medical research-Senior research fellowship (2021-2024)

Title: Targeting iron overload in Parkinson’s disease using a plant-derived chelator, mimosine

Authors: *H. P. J, R. A. SHENOI, G. CHANDRA, K. P. MOHANAKUMAR;
Inter university centre for biomedical research and SSH, Kottayam, India

Abstract: Brain iron dyshomeostasis caused by accumulation of labile iron, is implicated in Parkinson’s disease (PD). Excess labile iron can generate reactive oxygen species (ROS), contributing to oxidative stress-mediated neuronal cell death. Hence, chelating the labile iron pool in the brain is considered a promising therapeutic approach for PD. In pursuit of a safe and efficacious brain iron chelator, we focused our study on mimosine a plant-derived iron chelator that has not been previously explored for its ability to chelate brain iron. The iron-chelating and antioxidant activities of mimosine were evaluated using ferrozine and ferric reducing antioxidant power assays, where it exhibited approximately 80% iron chelation activity. Cell viability studies using MTT assay revealed that mimosine was non-toxic to SH-SY5Y neurons up to 45 μM for 48 h. Pretreatment with mimosine (10-45 μM) significantly improved cell viability following exposure to MPP^+ (2 mM), suggesting a potential neuroprotective effect against MPP^+-induced toxicity. We further examined the iron chelating ability of mimosine in an in *vitro* iron overload model, where SH-SY5Y cells were treated with ferric ammonium citrate (100 μM), followed by quantification of labile iron levels using ferene assay. Pretreatment with 50 μM mimosine

resulted in a 12.5% reduction in labile iron levels. Additionally, we evaluated the effects of mimosine and the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on striatal dopamine levels in C57BL/6 male mice using high-performance liquid chromatography with electrochemical detection. Dopamine levels in the control group were 56.86 ± 2.61 pmol/mg striatum, whereas the MPTP-treated group showed a significant reduction to 13.05 ± 4.17 pmol/mg ($p < 0.05$). In contrast, the mimosine-treated group maintained dopamine levels at 41.83 ± 8.42 pmol/mg, with no statistically significant difference from the control group. These results suggest that mimosine does not adversely affect dopaminergic tone and may exert a protective effect against dopamine depletion. Collectively, these findings support the potential of mimosine as a brain-permeable iron chelator. Further in vivo studies are warranted to validate its therapeutic efficacy in iron overload associated with Parkinson's disease

Disclosures: H.P. J: None. **R.A. Shenoi:** None. **G. Chandra:** None. **K.P. Mohanakumar:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.12/E38

Topic: C.03. Parkinson's Disease

Support: Supported by Beijing Life Science Academy (BLSA), No: 2024500CC0240

Title: Nicotine'S neuroprotective role in the mptp-treated mouse model of parkinson's disease via the gut-brain axis

Authors: R. LIU[1], ***Y.-N. MA**[1], W. MU[1], X. LI[1], Y. LI[1], J. ZHANG[1], Z. NI[1], Q. LI[1], H. CHEN[2,1], H. HOU[1];
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Abstract: Parkinson's disease (PD), the second most common neurodegenerative disorder, predominantly affects middle-aged and elderly populations. Its hallmark features include progressive motor deficits (e.g., bradykinesia and rigidity), degeneration of dopaminergic neurons in the substantia nigra, neuroinflammation, and dysbiosis of the gut microbiota. Although nicotine and its metabolites are associated with neuroprotective effects, the specific roles of its stereoisomers and primary metabolite remain poorly defined. Using an MPTP-induced PD mouse model, we investigated the therapeutic effects of S-nicotine, R-nicotine, and cotinine. All three compounds significantly improved motor function in both the rotarod test and gait assessments, though the degree of improvement varied between assays. Immunohistochemical and western blot analyses revealed preservation of tyrosine hydroxylase

(TH)-positive neurons, indicating protection of dopaminergic neurons. Additionally, pro-inflammatory cytokines levels were markedly reduced in central, peripheral and systemic compartments, indicating broad anti-inflammatory effects. 16S rRNA gene sequencing showed partial restoration of gut microbial diversity and composition, implicating gut-brain axis modulation in the observed neuroprotection. To further investigate the role of gut microbiota, we plan fecal microbiota transplantation (FMT) from treated donors to MPTP mice, which will clarify whether microbial remodeling drives or augments therapeutic outcomes. Collectively, our findings indicate that S-nicotine, R-nicotine, and cotinine confer neuroprotection in PD via a multi-system mechanism involving dopaminergic preservation, immune modulation, and gut microbiota regulation. These results highlight their promise as multi-target therapeutic candidates for PD.

Disclosures: R. liu: None. **Y. Ma:** None. **X. Li:** None. **J. Zhang:** None. **H. Chen:** None. **H. Hou:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.13/E39

Topic: C.03. Parkinson’s Disease

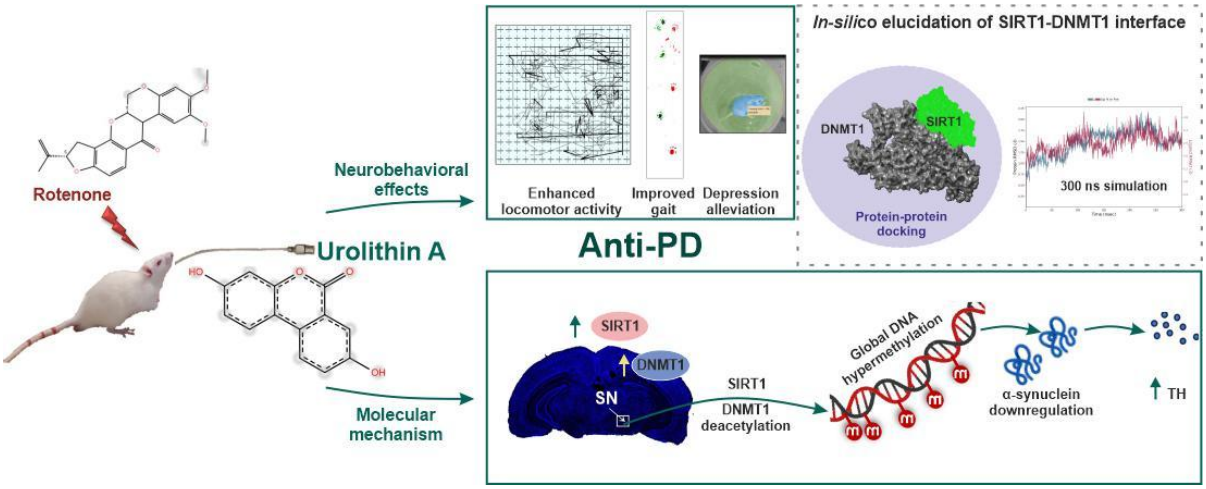
Support: NIPER JEE (Ph.D.) Scholarship

Title: Urolithin A Reverses Intranigral Rotenone-Generated Parkinsonism by Modulating α-Synuclein DNA Methyltransferase 1 Axis in Rats

Authors: *A. DEVI;
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Abstract: Epigenetic aberrations play a key role in the neurobiology of Parkinson’s disease (PD). Herein, we explored the post-translational changes of DNA methyltransferase 1 (DNMT1), an epigenetic marker, in a rotenone model of PD. Rats infused with intranigral rotenone showed an impaired locomotor activity and motor coordination in open field, rotarod, and gait assays. We also noted a depression-like phenotype in forced swim test (FST). These rotenone-generated motor and non-motor aberrations were reversed following peroral administration of urolithin A (UA) at 50 and 100 mg/kg doses. At molecular level, decreased mRNA/protein expression of NAD+-dependent sirtuin 1 (SIRT1) enzyme in substantia nigra (SN) was seen in rotenone-infused group. At the epigenetic level, we observed the decreased expression of DNMT1 and upregulated levels of acetylated DNMT1 (ac-DNMT1) in SN of rotenone-recipient rats. UA treatment elevated SIRT1 expression and DNMT1 deacetylation in the SN of rotenone-lesioned rats. The DNMT1 mRNA expression remained unchanged across the groups. The in-silico

binding of catalytic region of SIRT1 to DNMT1 (over BAH2 and catalytic domain) and stability over 300 ns simulation indicated the importance of SIRT1 in deacetylating the DNMT1 protein. This led to UA-mediated elevation of 5-methylcytosine (5-mC) levels resulting into global DNA hypermethylation in the SN. UA treatment downregulated the synuclein alpha (SNCA) gene expression and thus decreased the α-synuclein levels in rotenone lesioned rats. These findings indicate that UA treatment may reverse rotenone induced PD progression via modulation of SIRT1/ac-DNMT1/5-mC axis in the neuroanatomical framework of SN.



Disclosures: A. Devi: None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.14/E40

Topic: C.03. Parkinson's Disease

Support: The Sherman Foundation
Paula and Rusty Walter and Walter Oil & Gas Corp Endowment.

Title: Targeting Novel Mechanisms in Parkinson's Disease: Mitochondrial Dysfunction, Genomic Instability, and Senescence Reversed by a Carbon-Based Nanozyme

Authors: *V. VASQUEZ[1], M. KODAVATI[1], J. MITRA[1], V. MALOJI RAO[1], A. KATIYAR[1], T. KENT[2,3,4], M. L. HEGDE[1,5];
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Inst., Houston, TX; [4]Dept. of Chem., Rice Univ., Houston, TX; [5]Dept. of Neurosci., Weill Cornell Med. Col., New York, NY

Abstract: Emerging research in Parkinson's disease (PD) has identified a complex interplay of pathological mechanisms that extend past the classical features of dopaminergic neuron loss and alpha-synuclein (α-Syn) aggregation. These include progressive mitochondrial dysfunction, persistent genomic instability, and cellular senescence, all of which contribute to neuronal vulnerability and drive disease progression. Iron dyshomeostasis in PD-affected brain acts as a central amplifier, intensifying oxidative stress, DNA damage, and α-Syn aggregation, thereby compounding neurodegenerative processes. Our recent studies reveal loss of mitochondrial outer membrane translocase (TOM40) protein in PD patient brains, patient iPSC lines, and in vivo models, which was dependent on α-Syn oligomer accumulation, and results in a myriad of mitochondrial dysfunction (Vasquez et al, 2025). To counteract these interconnected and unexplored mechanisms, we employed a multifunctional carbon-based nanozyme conjugated with deferoxamine (DEF-OAC-PEG) for therapeutic application in PD. This nanozyme, which was originally developed by Kent and colleagues to prevent cellular toxicity from degraded blood products following intracerebral hemorrhage, exhibits multi-functional catalytic activity, including NADH oxidation to NAD^+, superoxide dismutase mimicry, and iron chelation, targeting key PD-related pathologies. In PD patient-derived dopaminergic neurons with SNCA gene triplication, DEF-OAC-PEG treatment restored levels of the mitochondrial outer membrane protein TOM40, improved cell viability following 6-hydroxydopamine (6-OHDA) exposure, reduced γH2AX DNA damage foci, preserved mitochondrial DNA integrity, and lowered phosphorylated α-Syn (pS129-α-Syn) levels in both SNCA triplication and toxin-induced neuronal models. In vivo, intraperitoneal administration of DEF-OAC-PEG in GBA1 KI x hSNCA mice at early disease stages (8 months) led to reduced expression of senescence-associated β-galactosidase and pS129-α-Syn in the cortex and midbrain. Immunofluorescence confirmed successful nanozyme delivery to $MAP2^+$ neurons, and treatment correlated with increased TOM40 protein levels. These findings illuminate critical, yet under-targeted, mechanisms in PD pathology and provide compelling in vitro and in vivo evidence for the therapeutic potential of DEF-OAC-PEG. This research was funded in part by the Sherman foundation as well as the Paula and Rusty Walter Oil & Gas Corp Endowment.

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Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.15/E41

Topic: C.03. Parkinson's Disease

Support: MJFF Grant 177733
NINDS Grant R44NS124398
NIGMS Grant R44GM121117

Title: Computational identification of a small molecule that enhances L-DOPA efficacy while reducing dyskinesia in Parkinson's disease through modulation of RIIβ subunit containing protein kinase A

Authors: *A. BORDBAR[1], B. BLOEM[1], C. LLOYD[1], T. H. JOHNSTON[2], J. S. SCHNEIDER[2], E. BEZARD[3], E. Y. PIOLI[3], Q. LI[4], J. WU[5], M. P. HILL[2], J. M. BROTCHIE[2], F. HERBERG[6], S. S. TAYLOR[5], D. M. WEINER[7], K. MERCHANT[8], I. FAMILI[1];
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Abstract: L-DOPA remains the gold standard for Parkinson's disease (PD), but its use is limited by motor complications including L-DOPA-induced dyskinesia (LID) and motor fluctuations. To overcome these challenges, we developed a transcriptomics-driven approach to disentangle L-DOPA's therapeutic and dyskinetic effects. By analyzing published striatal gene expression in a mouse model of PD using multivariate analysis, we identified distinct molecular signatures associated with the antiparkinsonian and pro-dyskinetic effects of L-DOPA. We compared these signatures against large-scale drug-induced gene expression databases to identify compounds that could mimic the beneficial effects of L-DOPA while counteracting LID-associated molecular changes. This virtual screen led to the identification of SB-0107, a positive allosteric modulator of protein kinase A (PKA) holoenzymes containing the regulatory subunit RIIβ. In the 6-OHDA lesioned rat model of PD, we found SB-0107 enhanced L-DOPA's efficacy while suppressing well-known LID-associated gene expression. Next, SB-0107 and novel deuterated analogs with improved pharmacokinetics were evaluated in three studies with MPTP-lesioned macaques with varying L-DOPA doses to induce variable expression of parkinsonian disability and LID severity. The compounds robustly and consistently improved parkinsonian disability and reduced dyskinesia, supporting the compound's potential as a dual-action adjunct to L-DOPA. PD and dopaminergic treatments are associated with changes in cognitive function. Thus, we evaluated SB-0107 in the chronic low-dose MPTP-lesioned macaque model of cognitive deficits in PD. Across two tasks sensitive to working memory, attention, discrimination, and reversal learning, the compound showed no negative effects under any condition and significantly improved performance on cognitive tasks under some conditions. Finally, we performed follow-up studies to characterize SB-0107's mechanism. First, striatal gene expression changes in 6-OHDA lesioned rats due to SB-0107 administration demonstrate modulation of genes downstream of PKA. Second, pharmacological assays showed no hits for known PD/LID targets and no off-target liabilities. Finally, computational docking revealed a novel binding site on the PKA RIIβ subunit. Together, these findings demonstrate a novel computational and phenotypic drug discovery strategy focused on dissociating therapeutic efficacy from side effects. Our results support a promising therapeutic candidate that enhances the benefits of L-DOPA while suppressing dyskinesia, offering a dual-action approach for improving PD treatment.

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Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.16/E42

Topic: C.03. Parkinson’s Disease

Support: Canada Foundation for Innovation
Genome Canada
NSERC
Alberta Innovates
Mitacs

Title: Lipidomic profiling across the Lewy body disease spectrum reveals distinct biomarker signatures

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Abstract: Background: Lewy body diseases (LBD), ranging from Parkinson's disease (PD) to Dementia with Lewy bodies (DLB), span a clinical continuum characterized by motor dysfunction and cognitive decline. Emerging evidence highlights a critical role for lipid metabolism in LBD pathogenesis. Blood-based lipidomic biomarkers may support improved diagnosis and therapeutic development. The Comprehensive Assessment of Neurodegeneration and Dementia (COMPASS-ND), a pan-Canadian cohort study led by the Canadian Consortium on Neurodegeneration in Aging (CCNA), offers a unique opportunity to explore lipidomic differences across the LBD spectrum. Methods: Serum samples and clinical data from COMPASS-ND participants were analyzed, including cognitively unimpaired (CU; n=56), PD without cognitive impairment (n=54), PD with mild cognitive impairment (PD-MCI; n=36), and Parkinson's disease dementia or DLB (PDD/DLB; n=33). Each group was randomly divided into discovery and validation cohorts for all pairwise cohort comparisons. Lipidomics analyses were performed using ultra-high-performance liquid chromatography coupled with high-resolution quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS). Samples underwent liquid-liquid extraction with isotopically labeled internal standards. Data processing and lipid identification were carried out using LipidScreener software. Identified lipids were normalized and subjected to multivariate (PCA, PLS-DA) and univariate statistical analyses. Significance was assessed using false discovery rate-adjusted volcano plots. Random forest models and ROC curve analyses were used to evaluate biomarker performance. Preliminary Results: Distinct lipidomic profiles were observed between CU and LBD groups, and among the LBD subtypes: PD vs. PD-MCI, PD-MCI vs. PDD, and PDD vs. DLB. Discriminatory models using panels of five lipids achieved area under the curve (AUC) values exceeding 0.90, indicating strong classification performance. Detailed results will be presented at the conference. Conclusion: Comprehensive lipidomic profiling reveals robust differences across the Lewy body disease spectrum, including between the closely related PDD and DLB subtypes. These findings suggest that specific lipid pathways are differentially affected with increasing cognitive impairment, supporting the potential for lipid-based biomarkers to aid in disease stratification and to uncover distinct pathogenic mechanisms within LBD.

Disclosures: S. Zhao: None. **R. Camicioli:** None. **K. Nelles:** None. **L. Li:** None. **R. Dixon:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.17/E43

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Syracuse University Start-up grant

Title: Restoring lysosomal acidification with nanoparticles mitigates A30P α-synuclein pathology in cellular and animal models of familial Parkinson's disease

Authors: *J. ZENG[1], M. REN[3], J. INDAJANG[4], K.-L. LIM[5], C. LO[2];
[2]Biol., [1]Syracuse Univ., Syracuse, NY; [3]Lee Kong Chian Sch. of Med., Lee Kong Chian Sch. of Med. - Nanyang Technological Univ., Singapore, Singapore; [4]Biomed. Engin., Cornell Univ., Mechanicville, NY; [5]Nanyang Technological Univ., Singapore, Singapore

Abstract: Parkinson's disease (PD) is an age-related neurodegenerative disorder marked by the toxic accumulation of α-synuclein (αSyn) in Lewy bodies and the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to motor impairments. A growing body of evidence implicates lysosomal acidification dysfunction as a key driver of αSyn accumulation, due to impaired autophagy-lysosomal degradation. Disruption in lysosomal pH compromises the activity of lysosomal hydrolases and contributes to defective clearance of misfolded proteins, including αSyn aggregates. While therapeutic strategies that restore lysosomal acidity using acidifying nanoparticles have shown promise in neurotoxin-induced or αSyn fibril-seeded models of sporadic PD, their application in familial PD models remains unexplored. In this study, we report the first use of poly(ethylene tetrafluorosuccinate-co-succinate) (PEFSU)-based acidic nanoparticles (AcNPs) in A30P αSyn-overexpressing SH-SY5Y cells and animal models of familial PD. In the cellular model, AcNPs successfully restored lysosomal acidification, reactivated autophagic flux, enhanced mitophagy and mitochondrial function, and protected against A30P αSyn-induced cytotoxicity. In Drosophila, AcNP treatment promoted αSyn clearance, preserved dopaminergic neurons, and improved motor performance. Moreover, in A30P αSyn transgenic mice, we observed significant downregulation of lysosomal V-ATPase subunits, indicating compromised lysosomal acidification, and are currently evaluating whether AcNPs can rescue these defects in vivo. Together, these results highlight lysosomal acidification dysfunction as a pathogenic mechanism in familial synucleinopathy and support AcNPs as a potential therapeutic platform for PD and related proteinopathies.

Disclosures: J. Zeng: None. **M. Ren:** None. **J. Indajang:** None. **K. Lim:** None. **C. Lo:** None.

Poster

PSTR067: Parkinson's Disease Therapeutic Strategies Using Small Molecule Therapeutics in Preclinical Animal Models

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR067.18/E44

Topic: C.03. Parkinson's Disease

Support: European Research Council No. #951294

Title: In vivo targeting of alpha-synuclein fibrils by specific nanobody in mice

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Abstract: Synucleinopathies are a group of neurodegenerative diseases caused by the intracellular accumulation of misfolded α-synuclein. Diagnosing synucleinopathies involves a combination of clinical evaluation, imaging, and pathological examination, with a diagnosis often made late, with symptoms well-advanced. Although symptom alleviation might be effective (e.g. Parkinson's disease), there is today no cure for combating the progression of synucleinopathies. Passive vaccination with antibodies is an actively pursued strategy. Among those, an emerging strategy involves nanobodiestargeting α-synuclein aggregates. Nanobodies are smaller and more stable than conventional antibodies, allowing them to penetrate the blood-brain barrier efficiently. They could be engineered to bind α-synuclein aggregates to promote their specific clearing for diagnostic and therapeutic purposes. This pilot feasibility study aims at *in vivo* imaging α-synuclein-specific recombinant nanobodies raised against various epitopes in an α-synuclein pre-formed fibril (PFF) mouse model. Using fluorescence optical imaging, we longitudinally follow in the same mice, for 4 months on the one hand, the intracerebrally delivered PFF, and on the other hand, the intraperitoneally administered nanobodies. B6 *albinos* male mice were injected with PFF labelled with IVISense 800 nm dye in the striatum. Two or four months after the model induction, the mice received an intraperitoneal injection of nanobodies against α-synuclein fibrils labelled with IVISEnse 680 nm dye. Seventy-two hours after the nanobodies injection, *ex vivo* imaging and immunofluorescence on brain slices were carried out.We show the *in vivo* follow-up of the PFF over time. After the intraperitoneal injection, nanobodies biodistribution revealed intense kidney staining and a hot spot in the brain that matched the PFF delivery. The *ex vivo* imaging confirms the co-localisation. Immunofluorescence against P(Ser129)-α-synuclein plus direct detection of the nanobodies by confocal imaging revealed an accumulation of the nanobodies in the striatum in the area of the PFF injection, with also detection of both P(Ser129)-α-synuclein and nanobodies in the *substantia nigra* following the spread of misfolded α-synuclein.Here, we pioneer the *in vivo* targeting and monitoring of α-synuclein fibrils by specific nanobodies using optical imaging. The data suggest that these nanobodies could be used therapeutically to combat synucleinopathies.

Disclosures: C. mazzocco: None. **N. Vaikath:** None. **M. Landry:** None. **C. Genevois:** None. **O. El-Agnaf:** None. **E. Bezard:** A. Employment/Salary (full or part-time): Motac France

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

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Program #/Poster #: PSTR068.01/E45

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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NINDS R21NS140565
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NIMHD LRP

Title: Choroid plexus volume alterations following extensive exposure to repetitive head impacts

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Abstract: Repeated head impacts (RHI) may impair the brain's ability to clear waste, leading to the buildup of hyperphosphorylated tau (p-tau) in perivascular regions—a defining characteristic of Chronic Traumatic Encephalopathy (CTE). The choroid plexus, which plays a key role in clearing neurotoxins from the brain, is a promising region for studying mechanisms underlying

p-tau deposition. This research explores the impact of RHI on choroid plexus volume and assesses its connection to Traumatic Encephalopathy Syndrome (TES), the clinical diagnostic framework of CTE, and the levels of certainty for CTE pathology. MRI data were obtained from the DIAGNOSE CTE Research Project for manual segmentation of the choroid plexus of 170 former American football athletes and 54 unexposed asymptomatic controls. Volumetric comparisons were conducted between the football player and control groups. In addition, choroid plexus volumes were assessed between football players with and without TES, and across the spectrum of levels of certainty for CTE pathology. Group differences were analyzed using an ANCOVA, adjusting for age, race, body mass index, education, imaging site, and APOE $\varepsilon 4$ allele status. Former football players demonstrated a significantly greater choroid plexus volume in both the left and right hemispheres compared to controls (right: $F(1,214)=6.979$, $p=0.01$; left: $F(1,214)=5.047$, $p=0.02$). No significant volumetric differences were found between those with a TES diagnosis compared to those without (right: $p=0.143$; left: $p=0.563$). There were also no significant differences across levels of certainty for CTE pathology (right: $p=0.215$; left: $p=0.475$). Consistent with findings in other neurological disorders, increased choroid plexus volume may suggest that RHI exposure disrupts perivascular clearance systems.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.02/E46

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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BU ADRC Research Education Component Program
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Title: Aberrant DNA methylation associated with chronic traumatic encephalopathy in human postmortem cerebral cortical tissue

Authors: *K. BREEN[1,2,4], N. AYTAN[2,4], J. K. BLUSZTAJN[3], A. C. MCKEE[2,4,3], H. LIN[5], T. D. STEIN[2,3,4];
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Abstract: Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative tauopathy associated with repetitive head impacts (RHI), commonly observed in contact sport athletes and military personnel. The diagnosis of CTE is defined by hyperphosphorylated tau in neurons surrounding blood vessels at the depths of the cortical sulci, which most commonly first occurs in the dorsolateral frontal cortex (DLFC). CTE can only be diagnosed postmortem, and it is classified into stages I through IV based on the severity and location of pathology. The neuropathological hallmarks of CTE and its association to RHI exposure are well-established, however the underlying molecular mechanisms contributing to CTE onset and progression remain incompletely understood. DNA methylation is modulated by environmental factors, such as RHI, and provides an epigenetic mechanism regulating gene expression; thus, investigating the association between DNA methylation and CTE may point to genes involved in its pathogenesis. Reduced representation bisulfite sequencing was used to assess DNA methylation at single-nucleotide resolution in autopsy DLFC gray matter sulcus samples. Frozen DLFC samples from the Understanding Neurological Injury and Traumatic Encephalopathy (UNITE) brain bank were queried for this study. A total of 401 male participants were included (mean age at death of 70 years), all of whom played contact sports for an average of 14 years, and 298 (74%) were diagnosed with CTE. Linear regression models, adjusted for age and smoking history, were used to assess the association between CTE stage and methylation of CpG sites. A genome-wide significance (GWS) threshold of 5×10^{-8} was applied. Additionally, differentially methylated regions (DMRs) were identified (at least 100 base pairs long, containing at least three nominally significant CpG sites (p=0.05), with over 80% of CpG sites in the region reaching nominal significance). A total of 18 CpG sites reached GWS and all mapped to Eukaryotic Translation Initiation Factor 4H (*EIF4H*) on chromosome 7. *EIF4H* also contained a DMR with 110 CpGs within a CpG island of its promoter. Pathway analysis using g-Profiler for aberrantly methylated genes with a p-value < 0.005 for their association with CTE stage (n=671 genes) revealed transcription regulatory activities and generation of neurons as top GO terms. Overall, we identified aberrant DNA methylation in DLFC associated with CTE stage implicating an epigenetic mechanism related to CTE pathology and progression.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.03/E47

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Vetenskapsrådet
Parkinsonfonden
Hjärnfonden

Title: Utilizing CRISPR-activation for induction of autophagy in an in-vitro alpha-synuclein aggregation model

Authors: *E. ARVIDSSON, Arvidsson;
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Abstract: Parkinson's disease (PD) is the second most common progressive neurodegenerative disease world-wide with no curative treatments currently available, highlighting the need for novel therapeutic options. A major hallmark of PD is the aggregation of misfolded alpha-synuclein protein (α-syn), making this one of the main targets for therapeutic intervention. We hypothesized that endogenous upregulation of Tfeb, a master regulator of autophagy and lysosomal biogenesis, could halt the formation of pathological protein aggregates in a cell model setting. To address this, we developed an in-vitro cell assay for induced aggregation of α-syn. We also screened and selected single guide RNAs (sgRNA) for CRISPR-activation (CRISPRa) of Tfeb, using a nuclease-deficient Staphylococcus aureus Cas9 fused to a VPR activator domain. To establish an in-vitro aggregation model, C6 rat glioblastoma cultures stably expressing GFP or human α-syn-GFP were transfected with human α-syn pre-formed fibrils (PFFs) to induce aggregation. Quantification of extra- and-intranuclear GFP-positive aggregates was performed using FloIT, a previously documented FACS protocol optimized for the detection of small particles and protein aggregates in cell culture. FloIT analysis of α-syn-GFP cultures 24 hours post-PFF transfection detected over 250 GFP-positive events per 1000 nuclei in the nuclear fraction (n=3; mean ± SEM = 257.7±8.8), along with nearly 100 GFP-positive events per 1000 nuclei in the extra-nuclear fraction (n=3; 98±6.1). Using a in-vitro single guide RNA (sgRNA) screening assay previously established in our lab, we selected three separate dual-sgRNA combinations resulting in over 6-fold increased Tfeb promoter activity compared to scrambled control (n=3; 6.94±0.63). To confirm sgRNA functionality on endogenous gene regulation, lentiviral transduction of selected sgRNA was performed in wild-type C6 cultures, resulting in over 3-fold increase in endogenous Tfeb expression compared to scrambled control (n=3; 3.1±0.36). All samples were prepared as minimum three technical replicates. These results display the functionality of the assay to be used as a model of α-syn aggregation, and confirms the ability of the in-vitro screening protocol to select sgRNA for endogenous gene regulation using CRISPRa in a rat cell line. Combining these methods to induce autophagy in the

aggregation model will contribute to the development of CRISPRa technologies and provide new insights in novel treatment avenues for protein aggregation disorders, including Parkinson's disease.

Disclosures: E. Arvidsson: None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.04/E48

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: European Research Council No. #951294
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Title: Nanoscale remodelling of the brain extracellular space across neurodegenerative proteinopathies

Authors: A. LOVISOTTO[1], J. ESTAÚN-PANZANO[2], I. CALARESU[3], S. NANDI[4], Q. GRESIL[5], ***B. H. BIOULAC**[6], B. DEHAY[7], L. GROC[9], L. COGNET[10], V. U. NÄGERL[11], E. BEZARD[8];
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Abstract: The extracellular space (ECS) is a complex, dynamic network occupying about 20% of the brain, filled with a cerebrospinal fluid-like solution rich in extracellular matrix (ECM) molecules. Its nanoscale architecture is increasingly recognised as a key factor in modulating pathological protein spread, yet comparative analyses across neurodegenerative conditions are lacking. We employed high-resolution approaches—including single-particle tracking with quantum dots and carbon nanotubes, cryo-electron microscopy, and *in vivo* two-photon shadow imaging—to examine ECS remodelling in mouse models of α-synucleinopathy, amyloidopathy, and tauopathy. In α-synuclein-overexpressing mice, ECS diffusivity was elevated and correlated with local degradation of hyaluronan (HA) near reactive microglia. Enzymatic HA depletion in vivo increased extracellular permeability, reduced α-synuclein accumulation, and enhanced microgliosis, identifying HA as a key ECM component controlling ECS geometry, molecular mobility, and inflammation. Remarkably, intracellular α-synuclein inclusions altered ECS rheology even in the absence of neuronal loss, indicating that intracellular aggregation alone is

sufficient to modify ECS properties. In the amyloidopathy APP/PS1 mice, amyloid plaques exhibited a dense cellular border enclosing a permeable amyloid core, with enhanced diffusivity detected within and around plaques. This was accompanied by ECM disruption and variability in nanoparticle access depending on plaque phenotype. In a tau seeding model, areas of tau aggregation displayed locally elevated diffusion and marked ECM remodelling, including altered astrocytic proteoglycan uptake. Diffusion dynamics were strongly influenced by regional cytoarchitecture, with sharper gradients observed between cell-dense and cell-sparse zones, underlining the structural determinants of ECS behaviour. Collectively, our data show that ECS remodelling is a common and early hallmark across distinct proteinopathies, linked to both molecular aggregation and ECM reorganisation. These findings highlight the ECS as an active participant in disease, possibly in its propagation, and suggest that targeting ECM components may offer novel therapeutic strategies for slowing neurodegenerative spread.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.05/E49

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Development of rabbit monoclonal antibodies and pair-based assays to interrogate TDP43 dysfunction

Authors: *J. W. HIRSCHFELD[1], A. AIELLO[1], R. W. CHO[2];
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Abstract: TAR DNA-binding protein 43 (TDP43) is a ubiquitously expressed DNA/RNA binding protein belonging to the heterogeneous nuclear ribonucleoprotein (hnRNP) family. TDP43 plays many roles in RNA metabolism, including transcriptional regulation and exon splicing. Dysfunctional TDP43, driven by genetic mutations, hyperphosphorylation, ubiquitination, and cleavage forms insoluble aggregates in the cytoplasm of cells. These aggregates are pathological hallmarks in the brain and spinal cord of patients with amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and limbic predominant age-related TDP-43 encephalopathy (LATE). These inclusions are also present in some patients with other neurodegenerative diseases (NDDs), including Alzheimer's disease (AD), Chronic traumatic encephalopathy (CTE), and Parkinson's disease (PD). Two posttranslational modifications in particular that promote aggregation of TDP43 are dual phosphorylation events

at Ser403/Ser404 and Ser409/Ser410 (Jo et al., 2020; de Boer et al., 2020).TDP43 cytoplasmic mis-localization and aggregation leads to mRNA mis-splicing and altered protein expression levels downstream. TDP43 dysfunction promotes the inclusion of cryptic exons in a number of transcripts, including those for Stathmin-2, UNC13A, and HDGFL2. Altered splicing of Stathmin-2 and UNC13A produces premature stop codons within the mRNA, promoting its clearance and resulting in a reduction of protein expression levels. Inclusion of a cryptic exon in HDGFL2 mRNA leads to alternative translated HDGFL2 that includes cryptic exon-encoded neo epitopes. Neo epitope-containing HDGFL2 is detected in CSF and plasma, suggesting that it may be a biomarker for disease (Ma et al., 2022; Quadros et al., 2024; Irwin et al., 2024). Rather than inclusion of cryptic exons, TDP43 dysfunction may also alter protein expression levels. Neuronal pentraxin-2 (NPTX2) levels, for instance, are increased upon TDP43 nuclear clearance and cytoplasmic aggregation (Hruska-Plochan et al., 2024). In order to examine TDP43 dysfunction, we aim to develop a portfolio of research tools that specifically and sensitively detect TDP43 phosphorylation, aggregation, and alterations to proteins downstream. This portfolio includes reagents against total and phospho-TDP43, Stathmin-2, UNC13A, HDGFL2, and NPTX2. In addition to individual reagents, we aim to develop pair-based assays that are able to measure expression levels of TDP43 and proteins regulated downstream. The antibodies and assays generated and validated here can be leveraged to further characterize and study neurodegenerative diseases with TDP43 pathology.

Disclosures: J.W. Hirschfeld: A. Employment/Salary (full or part-time): Cell Signaling Technology, Inc. **A. Aiello:** A. Employment/Salary (full or part-time): Cell Signaling Technology, Inc. **R.W. Cho:** A. Employment/Salary (full or part-time): Cell Signaling Technology, Inc..

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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NIH Grant R01AG068581
NIH Grant RF1AG076122

Title: In situ proteomic profiling of TDP-43 aggregates in human FTLD-TDP brain tissue

Authors: D. MORDERER[1], M. C. WREN[1], C. L. SMITH[1], D. W. DICKSON[2], ***W. ROSSOLL**[3];
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Abstract: Background: Pathological aggregates of the RNA-binding protein TDP-43 are a defining hallmark of the amyotrophic lateral sclerosis and frontotemporal dementia disease spectrum (ALS/FTD), and a common co-pathology in Alzheimer's disease (AD) and related dementias. Due to technical limitations for the purification of detergent-insoluble protein aggregates, little is known about the composition of these proteopathic inclusions in human brain tissue. Method: To interrogate the composition of neuropathological lesions, we established high-resolution photo-biotinylation of proteins in human FFPE brain tissue in situ with the novel Microscoop opto-proteomics discovery platform (Syncell). Regions-of-interest were defined by immunofluorescence with phospho-TDP-43 (pTDP-43) antibody and artificial intelligence (AI) image segmentation, followed by photolabeling, affinity purification, and mass spectrometry. Proteins associated with TDP-43 aggregates in each studied case were defined by scoring individual protein association probabilities using the Mass Spectrometry Interaction Statistics (MiST) algorithm. Association of selected proteins with TDP-43 aggregates were further validated by co-expression of candidate proteins with aggregation-prone variants of TDP-43 in HEK293 cells, and by co-immunofluorescence of FTLD-TDP brain sections with specific antibodies. Result: Automated microscopy-guided photolabeling of proteins within pTDP-43-positive aggregates with the Microscoop instrument allowed us to identify numerous proteins associated with TDP-43 aggregates across 4 studied FTLD-TDP cases. Analysis of protein-protein interaction networks for these proteins revealed several interaction clusters containing proteins involved in intracellular trafficking. Association of TDP-43 with components of the endomembrane system was confirmed using in vitro models of TDP-43 aggregation and in post-mortem brain sections from FTLD-TDP cases. Conclusion: A novel in situ proteomics discovery platform allowed us to profile TDP-43 aggregates in FTLD-TDP brains. The discovery that intracellular trafficking proteins are sequestered into these inclusions suggests that defects in endomembrane system functioning may contribute to the disease process in TDP-43 proteinopathies.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.07/E51

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Cryo-em structures of filaments from the brainsof individuals with variants g51d and h50q in alpha-synuclein

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Abstract: Gene dosage and point mutations in *SNCA*, the α-synuclein gene, give rise to familial forms of Parkinson's disease and dementia with Lewy bodies; an insertion mutation in *SNCA* causes juvenile-onset synucleinopathy. We previously reported the electron cryo-microscopy (cryo-EM) structures of α-synuclein filaments from the brains of individuals with Parkinson's disease, dementia with Lewy bodies and multiple system atrophy, as well as from the brain of an individual with juvenile-onset synucleinopathy. Here we report the cryo-EM structures of α-synuclein filaments from the frontal cortex of two cases with Parkinson's disease and mutation G51D and those from the amygdala of a case of Parkinson's disease and variant H50Q in α-synuclein. The G51D fold of assembled α-synuclein consists of two identical protofilaments with the Lewy fold with island B, but without the previously identified disconnected density island A. The protofilament interface is made of residues E46, V48 and H50. Filaments with the H50Q variant comprise a single protofilament with the Lewy fold and islands A and B. Unlike G51D, the pathogenicity of H50Q has been questioned. It remains to be seen if dimerisation of the Lewy fold may also underlie the pathogenicity of other missense mutations in α-synuclein. Moreover, the Lewy fold has a right-handed helical twist, while the G51D, multiple system atrophy and juvenile-onset synucleinopathy folds are left-handed, which may also be significant.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.08/E52

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Evaluating Plasma Levels of Alpha-Synuclein and Phosphorylated Alpha-Synuclein in Neurodegenerative Disorders Using SIMOA Technology

Authors: *S. TETRAULT;
Quanterix, Topsfield, MA

Abstract: Title: Evaluating Plasma Levels of Alpha-Synuclein and Phosphorylated Alpha-Synuclein in Neurodegenerative Disorders Using SIMOA Technology **Authors: George Kannarkat[1,2], Magdalena Brylska[3], Chengyuan Song[3,4], Shubhankar Nath[5], Kishore Malyavantham[5], Kelvin Luk[3,4] 1 Department of Neurology, University of Pennsylvania Perelman School of Medicine 2 Department of Neurology, Corporal Michael J. Crescenz, Department of Veterans Affairs Medical Center 3 Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine 4 Center**

for Neurodegenerative Disease Research, University of Pennsylvania Perelman School of Medicine 5 Quanterix Corp Abstract: The accumulation of misfolded and post-translationally modified alpha-synuclein, particularly its phosphorylated form at serine 129, is a hallmark of neurodegenerative disorders such as Parkinson's disease and Dementia with Lewy bodies. This study aims to measure the plasma levels of total alpha-synuclein and pSer129 alpha-synuclein in various cohorts, including healthy controls, Parkinson's disease patients, and individuals diagnosed with Lewy Body Dementia, as well as mouse models of synucleinopathy. Utilizing highly sensitive Single Molecule Array (SIMOA) technology, we assessed these biomarkers in plasma samples to determine their potential roles as diagnostic indicators and disease progression markers. Our approach allows for an unprecedented sensitivity in measuring these biomarkers, providing insights into their relevance in the pathophysiology of neurodegenerative diseases. We report here the patterns of alpha-synuclein and phosphorylated alpha-synuclein levels across the different cohorts and discuss how these findings can inform our understanding of alpha-synuclein's role in neurodegeneration and their potential application in clinical settings as a biomarker for diagnosis or monitoring therapeutic efficacy. We anticipate that our results will pave the way for better subject selection and monitoring in future studies aimed at targeting alpha-synuclein in neurodegenerative disease treatment.

Disclosures: S. tetrault: None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.09/E53

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Extracellular vesicles in blood samples may be used for synuclueinopathy biomarker development and diagnosis

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Abstract: This study investigates the value of neuron-derived extracellular vesicles (NDEs) and oligodendrocyte-derived exosomes (ODEs) for the diagnosis and monitoring of Multiple System Atrophy (MSA), a progressive neurodegenerative disorder. NDEs that carry neuron-specific proteins have emerged as a valuable source of biomarkers for the diagnosis and monitoring of neurodegenerative diseases, critically important for the development of disease-modifying treatments (DMTs) and in patient care. MSA is a type of synucleinopathy which presents significant diagnostic challenges due to strong phenotypic overlap with other neurodegenerative diseases. We have used ExoSORT, a proprietary immunoaffinity platform to isolate NDEs and ODEs from plasma samples from 65 MSA patients and 23 healthy controls (PROSPECT-UK study). We have analyzed nine EV biomarkers and neurofilament light chain (NFL), an

established marker of neurodegeneration. The comparison between both NDEs and ODEs from MSA and HC plasma revealed significant differences for three major EV markers (tetraspanins) and NFL. Notably, in ODEs, the comparison between MSA and HC groups using the combination of NFL and alpha-synuclein (α-Syn) achieved an Area Under the Curve (AUC) of 0.997, suggesting high accuracy of separation between disease and control. These findings indicate strong potential of EV biomarkers for precision MSA diagnosis and warrant further investigation in larger cohorts, including comparison with other synucleinopathies. We are also developing a novel Luminex-based assay to measure EV-associated aggregated α-Syn which shows promise for the quantitative assessment of synucleinopathies such as Parkinson's disease.

Disclosures: **A. Gekas:** A. Employment/Salary (full or part-time): full. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options. **E. Eitan:** A. Employment/Salary (full or part-time): full. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity, options. **S. Rau:** A. Employment/Salary (full or part-time): full. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.10/E54

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: R01NS118760
R01AG082352
U01NS112010

Title: Reproducibility of RT-QuIC of alpha-Synuclein and the Differentiation of Resultant Fibrils using Commercially Available Reagents

Authors: *A. VARGHESE, C. B. BARGAR, S. CHEN;
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Abstract: Alpha-synuclein (a-syn) protein has been shown to misfold and aggregate in multiple neurodegenerative disorders including Parkinson's disease (PD), multiple systems atrophy (MSA) and dementia with Lewy Body (DLB). Current developments in seed amplification technologies have utilized the prion-like propagation of a-syn aggregates as potential diagnostic biomarkers for these synucleinopathies. Yet, many of these developments are still in search of optimal protocols to ensure reproducibility. Here, we employed the real-time quaking-induced conversion (RT-QuIC) seed amplification assay to determine the effectiveness of commercially

available a-syn monomers in detecting the presence of a-syn aggregates within autopsied skin and brain samples from affected patients. Our preliminary data suggests our protocol was able to differentiate between controls and synucleinopathies in both skin and brain homogenates. Furthermore, we found no significant difference in using multiple lots of recombinant monomeric a-syn from the same vendor, establishing the reliability of these monomer preparations. We then collected patient-derived a-syn fibrils from the RT-QuIC and examined their structure. We found that the structure between PD and DLB fibrils differed significantly from that of MSA. Specifically, MSA fibrils were significantly wider than those of PD and DLB. These structural differences were conserved throughout both skin and brain samples from different patients. These characteristics could be used to potentially differentiate between these synucleinopathies. Our findings reinforce RT-QuIC as a reliable method to detect a-syn biomarkers as a potential diagnosis of synucleinopathies.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

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Program #/Poster #: PSTR068.11/E55

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: JSPS KAKENHI Grant Number 24K10749

Title: Drug discovery for the treatment of frontotemporal lobar degeneration using a premature termination codon cell model

Authors: *M. HOSOKAWA[1,2], Y. MORITA[1], A. EGASHIRA[1], A. URANO[1], Y. KOGA[1], T. NISHINAKAGAWA[1], D. ISHIBASHI[1];
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Abstract: Frontotemporal lobar degeneration (FTLD) is the second most frequent neurodegenerative disorder among juvenile onset dementias after Alzheimer's disease. FTLD is thought to be caused by a mutation in the granulin gene that results in haploinsufficiency, halving the granulin protein. In approximately 40% of granulin mutations, a premature termination codon (PTC), occurs upstream of the normal termination codon. Translational readthrough-inducing drugs (TRIDs) are compounds that “read-through” the PTC and promote the synthesis of full-length proteins. If TRIDs can read-through the PTC and promote progranulin protein synthesis, even in small amounts, it could lead to the treatment of FTLD. Therefore, the purpose of this study was to generate cellular models for the discovery of new therapeutic agents for FTLD. DsRed-stop-GFP/Piggy Bac plasmid was transfected into SH-

SY5Y cells with Transposase, and after selection with Puromycin, the cells were cloned by limiting dilution and a cell line was established. The cells emit green fluorescence because the termination codon is skipped when TRIDs are added. Dimethyl sulfoxide (DMSO), Amlexanox, and the new drug candidate were added to the cells at a final concentration of 10 μM/well. After 7 days of incubation, flow cytometry analysis was performed. The fluorescence intensities of the compound-added group and DMSO were compared, and those with increased GFP expression due to the addition of compounds were considered positive for read-through activity. In addition, a secondary screening of those compounds was performed. Using this cell model, 100 compounds were screened, and 12 compounds showed increased GFP expression compared to Amlexanox. Secondary screening of these 12 compounds revealed that 4 compounds clearly enhanced GFP fluorescence. These compounds were considered to be potential therapeutic agents not only for FTLD but also for other genetic diseases in which PTC appears. Future studies will examine whether these compounds can restore PGRN protein levels in the brains of granulin mutant mice.

Disclosures: M. Hosokawa: None. **Y. Morita:** None. **A. Egashira:** None. **A. Urano:** None. **Y. Koga:** None. **T. Nishinakagawa:** None. **D. Ishibashi:** None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

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Program #/Poster #: PSTR068.12/E56

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: 5R01NS136448-02
Roy J Carver Charitable Trust

Title: Incidence and Predictors of Early Alzheimer Disease Pathology in a State-wide Population Based Cohort

Authors: *M. HUNTER[1], **H. WILLIAMSEN**[2], M. M. HEFTI[2], S. PIERSON[2], K. FIOCK[2]; [1]Pathology, Univ. of Iowa, Iowa City, IA; [2]Univ. of Iowa, Iowa City, IA.

Abstract: Introduction: Although it is increasingly appreciated that Alzheimer disease tau pathology begins in subcortical structures, including the brainstem, the incidence of and risk factors for such pathology remain unclear. Most existing studies examine early AD pathology in older adults enrolled in clinical studies, which may not be representative of the population as a whole. We therefore present the first systematic assessment of neurodegenerative disease pathology in a large, population-based, statewide, predominantly rural, autopsy cohort.**Methods:** Consent for brain donation was sought from the families of all individuals who died in Iowa and came to autopsy at our institution or state medical examiner's office. We excluded individuals with known neurologic disease, and those whose brains were too damaged for examination. A

neurodegenerative disease workup was conducted on all cases, including staining for tau, beta-amyloid, alpha-synuclein, and TDP-43. Statistical analysis was done using Pearson's correlation coefficients and generalized linear models.**Results:** We included 118 total donors with a median age of 55 years (range 17-94). Tau pathology of any kind was rare before age 29 (3/11, all Braak Stage 0). Incidence of tau pathology in patients under age 40 was significantly lower in our cohort compared to a similar recently published Japanese study (29.6% vs. 71.4%). Most individuals under age 60 had tau pathology without concurrent beta-amyloid (30/38). Tau limited to the locus coeruleus was only seen in individuals between 30 and 60. Locus coeruleus tau pathology was not universal in those with tau pathology elsewhere. Tau pathology showed strong correlations between cingulate and middle frontal gyrus (r=0.676) and between amygdala and hippocampus (r=0.661). There was moderate correlation in the locus coeruleus and substantia nigra (r=0.426). Alpha-synuclein and TDP-43 pathology were seen almost exclusively in patients over 60 years old. Significant predictors of tau pathology in the locus coeruleus included age ($p<0.001$) and increased rurality of the county in which the individual's death occurred ($p=0.0022$). Sex, history of depression, and history of alcohol use disorder were not significant predictors of tau pathology in the LC.**Conclusions:** Our findings suggest that the incidence of early tau pathology may, for unclear reasons, be lower in a North American than in East Asian populations. Our data also suggests that, in most patients, tau pathology begins to develop before age 50, commonly but not always in the locus coeruleus. In our demographic data, only age and rurality were predictive of tau pathology in the locus coeruleus.

Disclosures: M. Hunter: None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.13/E57

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Ultra-high plex in situ spatial proteomic profiling of tau neuropathology across human tauopathies: progressive supranuclear palsy, corticobasal degeneration and Alzheimer's disease

Authors: *S. KOUTARAPU[1], K. ROBERTS[2], J. M. WALKER[5], T. ORR[3], T. THEISEN[6], M. VANDENBERG[7], C. WILLIAMS[6], A. HECK[6], C. L. WHITE, III[8], K. YOUNG[6], J. F. CRARY[9], M. HOANG[10], J. BEECHEM[11], M. ORR[4];
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Abstract: The intracellular deposition of phosphorylated tau proteins is a key feature of many neurodegenerative diseases, including Alzheimer's disease (AD), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD). Tau neuropathology includes intracellular tau deposition as neurofibrillary tangles (NFTs) within neurons, as well as tufted astrocytes, astrocytic plaques, and coiled bodies within glial cells. Tau deposition correlates with cognitive decline, motor dysfunction, and coordination abnormalities, and multiple, potentially interconnected, disease processes likely mediate toxicity. Unraveling the complex molecular changes occurring within a cell containing pathogenic tau and its influence on neighboring cells, cellular networks, and brain regions demands high resolution spatial solutions. Innovative approaches are needed to decipher how intracellular tau accumulation causes diverse symptomatology across tauopathies. Recent advances in spatial proteogenomic technologies allow for simultaneous quantitative analyses of protein and RNA at single cell levels. The GeoMx® Digital Spatial Profiler platform enables high-plex multiomics in discrete regions of interest (ROIs) from formalin-fixed paraffin-embedded (FFPE) postmortem brain tissues. Using antibodies and in-situ hybridization (ISH) probes coupled to photocleavable DNA barcodes and next generation sequencing (NGS), we employed the Human Neural Proteome Atlas (500+ targets), Human Immuno-Oncology Proteome Atlas (570+ targets), an ultra-high-plex neural proteomic panel, and a Whole Transcriptome RNA panel to examine neurons and glia with and without intracellular deposition of phosphorylated tau. This spatial multiomics approach was applied to the hippocampus to determine pathogenic differences between PSP, CBD, AD and controls. We identified shared and unique molecular signatures of cells and their microenvironments across tauopathies. Moreover, mapping the proteomics data back to the tissue revealed histological features (e.g., nuclear and cytoplasmic morphology, neighboring environment, and co-pathologies) associated with the molecular phenotypes. Lastly, we utilized the CosMx® Spatial Molecular Imager platform and a 68 plex protein panel tailored for dissecting neurodegenerative disease pathology to examine tissue wide differences in hippocampal neurons with single cell resolution. Together these data are advancing knowledge of disease heterogeneity across tauopathies and has potential to identify novel therapeutic targets, and guide precision medicine for tauopathies.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Ministry of Education Academic Research Fund (AcRF) Singapore Tier 2 (MOE-T2EP30122-0009)
Ministry of Education Academic Research Fund (AcRF) Singapore Tier 1 (FY2024-MOET1-0001)

Title: Parkinson's-linked Synaptojanin 1 Loss in Dopamine Neurons Triggers Synaptic Degeneration and Striatal TH Interneuron Compensation

Authors: *Y. LIN[1], B. MUKHERJEE[1], M. LIU[1], Z. LI[1], X. CAO[1], M. CAO[1,2];
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Abstract: Synaptic dysfunction is increasingly recognized as an early feature of Parkinson's disease (PD). Synaptojanin 1 (SJ1), a phosphoinositide phosphatase essential for synaptic vesicle recycling, is genetically linked to early-onset Parkinsonism (EOP). While SJ1-R258Q knock-in mice recapitulate EOP-like phenotypes, whole-body mutants introduce behavioral complexity that limits our understanding of SJ1's role within the dopamine (DA) system. To address this, we generated DA neuron-specific SJ1 conditional knockout (SJ1-DA cKO) mice. These mice exhibited severe dystrophy of striatal DA terminals and altered striatal DA metabolism, confirming SJ1's cell-autonomous role in midbrain DA neurons. Notably, despite profound synaptic deficits and reduced DA levels in the striatum, SJ1-DA cKO mice maintained normal motor behavior in standard motor tests. Potential local adaptation was observed through a robust emergence of induced tyrosine hydroxylase-positive interneurons (iTHINs) in the striatum. These iTHINs expressed DA markers such as aromatic L-amino acid decarboxylase (AADC), and formed apparent connections with DA terminals, as revealed by AAV-based labeling. Acute SJ1 deletion in the DA neurons of adult or aged mice showed similar synaptic pathology but fewer iTHINs, suggesting a developmental window for this plasticity. Together, these findings reveal a critical role for SJ1 in maintaining DA synaptic function and uncover an adaptive striatal response to DA loss, informing future therapeutic strategies for PD.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: National Institute of Health (NIH-NINDS, 1RF1NS110354-01)
Department of Defense Early Investigator Research award (W81XWH-19-1-0264)

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Parkinson's Foundation Research Center of Excellence (PF-RCE-1946)

Title: Synaptic vesicle endocytosis deficits underlie GBA-linked cognitive dysfunction in Parkinson's disease and Dementia with Lewy bodies

Authors: *V. D J[1,2], D. BÄCKSTRÖM[5,2], R. CHAKRABORTY[2], J. RUAN[3], P. MISTRY[3], S. S. CHANDRA[4];
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Abstract: *GBA* is the major risk gene for Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB), two common α-synucleinopathies with cognitive deficits. We investigated the role of mutant *GBA* in cognitive decline by utilizing Gba (L444P) mutant, SNCA transgenic (tg), and Gba-SNCA double mutant mice. Notably, Gba mutant mice showed robust cognitive deficits but lacked PD-like motor deficits or α-synuclein pathology. Conversely, SNCA tg mice displayed age-related motor deficits, without cognitive abnormalities. Gba-SNCA mice exhibited both cognitive decline and exacerbated motor deficits, accompanied by greater cortical phospho-α-synuclein pathology, especially in layer 5 neurons. Single-nucleus RNA sequencing of the cortex uncovered synaptic vesicle (SV) endocytosis pathway defects in excitatory neurons of Gba mutant and Gba-SNCA mice, via robust downregulation of genes regulating SV cycle and synapse assembly. Immunohistochemistry and electron microscopy validated these findings. Our results indicate that Gba mutations, while exacerbating pre-existing α-synuclein aggregation and PD-like motor deficits, contribute to cognitive deficits through α-synuclein-independent mechanisms, involving dysfunction in SV endocytosis.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

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Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NINDS U01NS093334
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NIMHD LRP

Title: A novel grading framework for cavum septum pellucidum in former contact-collision sport athletes

Authors: *J. STEARNS[1,4], M. ESTRADA[1,4], A. S. MIRMAJLESI[1,4], S. CANGE[1,4], D. MERCADO[1,4], A. WICKHAM[1,5], C. H. ADLER[7], C. BERNICK[8,9], L. J. BALCER[2,10,3], J. L. CUMMINGS[11], E. M. REIMAN[12,13,14,15], R. A. STERN[16,17,18], M. E. SHENTON[5,6,19], R. J. RUSHMORE, III[5,20], H. ARCINIEGA[1,4];
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Abstract: Repetitive head impacts (RHI) are associated with an increased risk for developing Chronic Traumatic Encephalopathy (CTE), a neurodegenerative disease observed in contact sport athletes. The cavum septum pellucidum (CSP), a nonspecific but frequently reported structural anomaly in CTE, has been commonly identified in postmortem examinations. In this study, we present a revised CSP grading framework specifically designed for athletes in contact-collision sports and validate it using a novel volumetric segmentation method combined with CSP length measures. This approach enhances grading objectivity and broadens its applicability for both clinical and research settings. Data from the DIAGNOSE CTE Research Project, focusing specifically on former American football players (n=170), was used to develop a revised CSP grading scale. The CSP grading scale is defined by the presence and progressive separation of the septum. Two independent raters evaluated CSP grade, volume, and length. Inter-rater reliability was measured using Cohen's Kappa, and a Kruskal-Wallis test was conducted to examine the relationship between CSP grades and both volume and length measurements. Inter-rater reliability was nearly perfect (Cohen's Kappa = 0.9, $p < 0.001$). Following consensus between raters, CSP grades were distributed as follows: Grade 0: 37/170 (21.8%), Grade 1: 36/170 (21.2%), Grade 2: 65/170 (38.2%), Grade 3: 9/170 (5.3%), and Grade 4: 23/170 (13.5%). Additionally, CSP volume and length significantly increased with higher grades, with all p's < 0.001. Our new CSP grading framework, validated with a novel volumetric segmentation method and CSP length measures, enhances assessment accuracy and reliability. This standardized approach advances CSP evaluation in contact-collision sport athletes, offering

valuable insights into RHI-related brain changes that may aid in assessing the consequences of RHI and diagnosing CTE during life.

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Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.17/F3

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

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NIH Grant T32 GM007250

Title: Cereblon homeostasis is perturbed by traumatic brain injury and critical for mitochondrial dynamics

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Abstract: Misfolded proteins and aggregates are implicated in neuropsychiatric diseases, with proper protein degradation crucial to mitigating pathology. Cereblon, a component of the E3 ubiquitin ligase complex, regulates neurodevelopment by targeting proteins for degradation. Cereblon mutations are associated with autosomal recessive mild intellectual disability, while its reduction leads to synaptic and cognitive deficits. Recently cereblon has been investigated in the context of neurodegeneration, demonstrating modulated levels across models of dementia-related disorders, with pharmacologic manipulation of cereblon activity proving therapeutic benefit. However, the mechanisms linking cereblon dysfunction to these outcomes remain unclear. To investigate the mechanistic impact of cereblon on neuronal health, we sought to measure cereblon levels in a mouse model of traumatic brain injury (TBI). We observed increased levels of cereblon one-year post-injury. To explore this finding, we generated cereblon deficient (KO) and overexpressing (KI) neurons, and applied an in vitro model of TBI to assess changes in cellular function. Our proteomic analysis identified mitochondrial function as a key hub of cereblon-mediated reaction to injury. Additionally, both cereblon KI and exposure to injury reflected similar deficits in mitochondrial activity, whereas cereblon KO was able to improve mitochondrial function. This pattern of mitochondrial activity was recapitulated when analyzing pathologic mitochondrial fission activity, with higher fission reflected in cereblon KI and injury, and lower in cereblon KO. We chose to then investigate the role of cereblon in vivo, generating a neuron-specific KO of cereblon in mice. We uncovered reduced neurogenesis and increased lipid peroxidation in our cereblon KO animals, indicating impaired brain health and heightened susceptibility to neurodegeneration. Our data indicate that upsetting the homeostatic balance of cereblon in either direction in the brain has deleterious effects on brain health. This identifies unique considerations of the challenges of altering cereblon levels to achieve therapeutic benefit.

Disclosures: S.G. Caradonna: None. **E. Miller:** None. **M. Byrne:** None. **R. Singh:** None. **S.J. Tripathi:** None. **S. Chakraborty:** None. **J.J. Higgins:** None. **J. Duong:** None. **P. Sridharan:** None. **E. Vazquez-Rosa:** None. **B.D. Paul:** None. **A.M. Rajadhyaksha:** None. **A.A. Pieper:** None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.18/F4

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Spatial transcriptomics reveals hippocampal region-specific molecular signatures implicated in neurological disorders

Authors: T. CAO[1], L. GUO[1], L. WANG[1], T. NGUYEN[2], T. PHAM[2], S. PHAM[2], L. CHEN[3], J. ZHOU[3], Y. WANG[3], W. LIAO[3], ***S. UDAY MAHAJANI**[3];
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Abstract: The hippocampus, located within the medial temporal lobe, plays a central role in learning, memory consolidation, and spatial navigation. Two distinct regions within the hippocampus, CA1 and CA3, exhibit differential vulnerability in neurological diseases like Alzheimer's disease (AD), epilepsy, schizophrenia, and age-related cognitive decline. The CA3 region has been reported to be more susceptible to seizure activity, hypoxic damage, and early tau pathology in AD. However, the molecular basis of this regional susceptibility remains poorly understood.Understanding the spatial organization of gene expression across these regions is essential for revealing how molecular heterogeneity underpins region-specific functions and pathological susceptibilities. Using a spatial transcriptomics workflow that combined Stereo-seq, DNBSEQ-T7 sequencing, and the SpatialX analysis platform, we profiled over 1.5 million cells in sagittal rat brain sections to identify 15 transcriptionally distinct clusters corresponding to anatomical brain regions.Focusing on spatial gene expression differences between CA1 and CA3, we identified a set of genes significantly enriched in CA1, including *Pou3f1*, *Nts*, *Fibcd1*, and *Lyzl4*, associated with synaptic signaling, neuropeptide activity, and innate immune modulation. On the contrary, CA3 showed selective enrichment of *Cpne4*, *Cst6*, *Nnat*, *Fxyd7*, associated with calcium signaling, protease inhibition, and ion homeostasis. These spatially anchored transcriptional differences provide new insight into how subregional gene expression programs may influence differential circuit vulnerability and progression of hippocampal pathology.Our findings highlight the Stereo-seq spatial transcriptomics platform's ability to map molecular heterogeneity across hippocampal architecture and uncover transcriptional patterns that may underlie disease-specific degeneration. We also present new candidate genes and pathways for investigating early-stage pathophysiology and region-targeted therapeutics in neurological disorders.

Disclosures: **T. Cao:** A. Employment/Salary (full or part-time): STOmics. **L. Guo:** A. Employment/Salary (full or part-time): STOmics. **L. Wang:** A. Employment/Salary (full or part-time): STOmics. **T. Nguyen:** A. Employment/Salary (full or part-time): BioTuring. **T. Pham:** A. Employment/Salary (full or part-time): BioTuring. **S. Pham:** A. Employment/Salary (full or part-time): BioTuring. **L. Chen:** A. Employment/Salary (full or part-time): Complete Genomics. **J. Zhou:** A. Employment/Salary (full or part-time): Complete Genomics. **Y. Wang:** A. Employment/Salary (full or part-time): Complete Genomics. **W. Liao:** A. Employment/Salary

(full or part-time): Complete Genomics. **S. Uday Mahajani:** A. Employment/Salary (full or part-time): Complete Genomics.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.19/F5

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Title: Thermal dynamics of prion and protein misfolding: a computational approach

Authors: *G. LLOYD;
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Abstract: Protein misfolding is a key feature of prion diseases, with higher temperatures accelerating the transition from the normal cellular form (PrP^{C}) to the pathogenic scrapie form (Prp^{Sc}). However, the exact relationship between temperature and the molecular dynamics of misfolding is not yet fully understood. In this study, we developed a computational model to examine how temperature drives the misfolding of prion proteins, taking into account important biophysical factors such as protein concentration, seed concentration, and thermodynamic parameters, including activation energy and conformational stability. Using temperature-sensitive rate constants based on Arrhenius kinetics, the model simulates the ratio of proteins that fold correctly versus those that misfold under varying thermal conditions. The simulation results reveal thresholds at which rising temperatures increase the proportion of misfolded species, aligning with experimental findings on heat-induced conformational transitions. This work provides a quantitative framework for investigating how heat stress disrupts protein homeostasis and triggers pathogenic misfolding in prion diseases. Future research should explore whether other biological systems can withstand the temperatures identified in this study; if these systems are compromised, it would limit the feasibility of utilizing such temperatures in anti-prion therapies.

Disclosures: G. Lloyd: None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.20/F6

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: ASBMB undergraduate research grant

Title: Insight into Synucleinopathies in a Yeast Model: Unravelling Molecular Determinants of β-Synuclein and γ-Synuclein Toxicity

Authors: *L. CASARES[1], H. KIERNAN[1], S. CHANDAVIMOL[2], F. BERTOLOTTI[4], S. GACEK[2], S. K. DEBBURMAN[3];
[1]Neurosci., [2]Lake Forest Col., Lake Forest, IL; [3]Biol., Lake Forest Col., lake Forest, IL; [4]Univ. of Chicago, Chicago, IL

Abstract: Synucleinopathies, a group of disorders characterized by the abnormal folding and aggregation of proteins from the synuclein family (including α-, β-, and γ;-synuclein), includes Parkinson's Disease (PD), the second most prevalent neurodegenerative condition. While α-synuclein's role in PD is well established, less is understood about the involvement of β- and γ;-synucleins in neurodegeneration and toxicity. However, two mutations in β-synuclein (P123H and V70M) are associated with Dementia with Lewy Bodies (DLB). In our Saccharomyces cerevisiae (budding yeast) PD model system, we recently discovered that β-synuclein is toxic but less than α-synuclein and γ;-synuclein is non-toxic. Here, we further expanded our evaluation of the toxicity potential of β- and γ;-synuclein. 1) To understand whether familial mutant toxicity was due to loss of the original amino acid or gain of the new mutant, we evaluated substitution mutants for V70M and P123H by changing the original amino acid with representative polar, nonpolar, basic, and acidic residues. For the V70 position, we found that the level of hydrophobicity was key for toxicity and aggregation. For the P123 position, we observed more evidence for the loss of the original proline than for the gain of the mutant basic amino acid. 2) We swapped the known familial mutations in α- and β-synuclein onto other synucleins at conserved locations. We found that α-synuclein familial mutations linked with amino acids A18, A29, A30, E46, G51, and A53 can differentially regulate β-synuclein toxicity, however, γ;-synuclein's non-toxicity remains unaltered with α-/β- familial swap mutations at A18, A30, E46, and V70. 3) We combined both V70M and P123H mutants and found their combinatorial toxicity to be additive. 4) We assessed the impact of altering those amino acids in β- and γ;-synucleins which are conserved post-translational modification sites from α-synuclein. We found that putative acetylation (K6), nitration (Y39), SUMOylation (K85) and phosphorylation (S118) sites in β-synuclein regulate its toxicity. In γ-synuclein, only putative acetylation site (K6) regulates its toxicity. 5) We also found that several synucleinopathy-linked environments modelled in genetically altered yeast strains can increase both β- and γ;;-synuclein toxicity. 6) Finally, we used AlphaFold to generate predicted protein models for all three synucleins, which show that β-synuclein can polymerize like α-synuclein (but requires at least seven monomer additions), while γ-synuclein never aggregates. This study highlights the usefulness of yeast and AI models in better understanding β- and γ-synuclein pathogenicity in neurodegeneration.

Disclosures: L. Casares: None. **H. Kiernan:** None. **S. Chandavimol:** None. **F. Bertolotti:** None. **S. Gacek:** None. **S.K. Debburman:** None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.21/F7

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: RF1 AG077103
R01 AG065628
R21 AG083638

Title: Shared organelle dysfunction among neurological disorders leading to Alzheimer's disease

Authors: *G. E. STUTZMANN[1], S. D. GINSBERG[2], W. GALLEGOS[3], D. F. STEINBRENNER[4], S. GUPTA[1], E. K. WEBBER[5], J. A. POTASHKIN[6];
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Abstract: The symptoms, histological features and major risk factors for Alzheimer's disease are deeply characterized and include cognitive decline, amyloid and tau aggregates, neuroinflammation and neurodegeneration. Despite decades of research devoted to this disease, remarkably little is understood regarding underlying pathophysiological mechanisms that initiate AD. However, with recent technology that expands the interrogation of clinically-based models, such as transcriptomics and human iPSC-derived brain cells from patient populations a consensus is forming around core pathways triggering AD. An additional strategy to identify AD drivers is to examine common physiological and genetic changes in conditions that greatly increase AD risk. Here, we selected traumatic brain injury (TBI; one of strongest environmental risk factors for AD) and Down syndrome (DS; the highest genetic risk factor for AD) to identify early mechanistic features shared with AD. By integrating human and mouse single cell transcriptomics and iPSC-derived neurons, we are synthesizing a genetic and pathophysiological model for early drivers of AD. Consistent with their overlap in differentially expressed gene pathways, key lines of convergence among TBI, DS, and AD point to an early and upstream dysregulation of intracellular Ca2+ homeostasis, mitochondrial dysfunction, endo-lysosomal deficits and synaptic deficits. Using a series of electrophysiological assays, live cell imaging of targeted biosensors, and immunoassays in human neurons derived from these patients and/or respective mouse models, we probed each of these core pathogenic hubs and compared across conditions to identify shared upstream mechanisms. We found that dysregulated Ca2+ release was a common essential component, and triggered pathogenic cascades driving amyloid and tau accumulation, as well as synaptic signaling alterations. Likewise, TBI and DS brains and neurons displayed amyloid and tau expression similar to AD. The excess intracellular Ca2+ underlies tau accumulation via impaired lysosome activity, as well as mitochondrial dysfunction and superoxide production, and synaptic defects associated with impaired memory encoding.

Disclosures: G.E. Stutzmann: None. **S.D. Ginsberg:** None. **W. Gallegos:** None. **D.F. Steinbrenner:** None. **S. Gupta:** None. **E.K. Webber:** None. **J.A. Potashkin:** None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.22/F8

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Start-up grant

Title: Restoration of lysosomal acidification attenuates propagation of metabolic inflammation along the body-brain axis

Authors: *X. CHEN[1,4], J. ZENG[2,4], C. LO[3,4];
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Abstract: Obesity leads to metabolic inflammation in disorders such as metabolic-associated fatty liver disease (MASLD), which are emerging as major risk factors for neuroinflammation and neurodegeneration. Recent studies suggest that autophagy and lysosomal impairments in key metabolic organs, including the liver and adipose tissue, contribute to systemic inflammation that may propagate from the periphery to the brain. In this study, we investigate whether restoring lysosomal acidification and associated metabolic functions in the liver and adipose tissue can reduce the secretion of tumor necrosis factor (TNF), a key inflammatory mediator, to mitigate neuroinflammation and neurodegeneration. We first conducted data mining of existing human RNA datasets and confirmed that MASLD patients exhibit dysregulation in biological pathways associated with autolysosomal dysfunction and TNF-mediated inflammation. In HepG2 liver cells and 3T3-L1 adipocytes, lipid overload via palmitate treatment impaired lysosomal acidification and increased TNF secretion, both of which were reversed by lysosome-acidifying nanoparticles (AcNPs). When conditioned media from AcNPs-treated, lipotoxic HepG2 or 3T3-L1 cells was applied to SH-SY5Y neuronal cells, TNF-induced necroptotic cell death was significantly reduced. In a high-fat diet (HFD) mouse model of obesity, we observed autolysosomal dysfunction and inflammation in both the liver and adipose tissues. These peripheral defects led to elevated circulating TNF levels that propagated to the brain, inducing neuroinflammation and neurodegeneration, characterized by TNF-driven necroptosis and cognitive decline, both of which were attenuated by intraperitoneal administration of AcNPs. This study underscores the role of peripheral metabolic inflammation, driven by autolysosomal dysfunction, in mediating obesity-induced neuroinflammation and neurodegeneration along the body-brain axis.

Disclosures: X. Chen: None. **J. Zeng:** None. **C. Lo:** None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.23/F9

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: SECIHTI GRANT 319578

Title: Disrupted chaperone-mediated autophagy and autophagic flux in familial Alzheimer's disease fibroblasts.

Authors: *M. CARDENAS-AGUAYO[1,2], N. LUMBRERAS-ZAVALA[2], M. SILVA-LUCERO[2];
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Abstract: Introduction. Alzheimer's disease (AD), the most common form of dementia, is neuropathologically defined by Amyloid-beta (Ab) plaques and neurofibrillary tangles containing hyperphosphorylated tau. AD cases are categorized as either sporadic late-onset (LOAD; >99%) or familial early-onset (FAD/EOAD; 1%), the latter driven by autosomal dominant mutations in APP, PS1, or PS2. In PS1-mutant FAD fibroblasts, our group and others have reported dysregulated macroautophagy, disrupted stress-response pathways, and aberrant tau phosphorylation (Lopez-Toledo et al., 2022). Since both macroautophagy and chaperone-mediated autophagy (CMA) are implicated in neurodegeneration, we evaluated their roles in FAD patient-derived fibroblasts. **Methods.** Skin fibroblasts from FAD patients and unaffected controls were obtained from the Coriell Institute (New Jersey) and cultured in Eagle's MEM supplemented with 15% non-inactivated FBS. Macroautophagy and CMA markers were analyzed by Western blotting. To assess autophagic activity, cells were subjected to nutrient deprivation (fasting, cultured in the absence of FBS) and chloroquine treatment, followed by evaluation using the Cyto-ID assay (ENZO autophagic detection kit) and the Premo™ Autophagy Tandem Sensor (Thermo Fisher autophagic flux detection system). **Results.** Autophagy assays revealed distinct dysregulation in FAD fibroblasts compared to healthy controls. Premo™ Autophagy Tandem Sensor analysis demonstrated significantly reduced autophagic flux in FAD cells, despite elevated LC3-II levels, indicating increased autophagosome formation despite a proper degradation of the cargo. Western blot analysis of CMA markers showed consistent downregulation of both LAMP2A and Hsc70 in FAD fibroblasts, suggesting concomitant impairment in chaperone-mediated autophagy. **Conclusion.** Fibroblasts from FAD patients exhibited significant dysregulation in both autophagy activity and CMA markers compared to healthy controls. These findings in peripheral cells may facilitate drug screening, early disease detection, and the identification of novel biomarkers for Alzheimer's disease.

Disclosures: M. Cardenas-Aguayo: None. **N. Lumbreras-Zavala:** None. **M. Silva-Lucero:** None.

Poster

PSTR068: Cellular and Molecular Mechanisms in Tauopathies, Synucleinopathies, and Related Diseases

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR068.24/F10

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: Alzheimer's Association International Research Grant
Syracuse University Start-Up Grant

Title: Defective lysosomal acidification contributes to TNF-TNFR1 mediated neuronal death in Alzheimer's disease

Authors: *C. LO[1], J. ZENG[2], J. INDAJANG[3], R. REYNOLDS[4], A. M. BARRON[5]; [1]Biol. and Neurosci., [2]Syracuse Univ., Syracuse, NY; [3]Cornell Univ., Ithaca, NY; [4]Imperial Col. London, London, United Kingdom; [5]Nanyang Technological Univ., Singapore, Singapore

Abstract: Tumor necrosis factor (TNF) receptor 1 (TNFR1) signaling mediates neuronal necroptosis in Alzheimer's disease (AD). Interaction of TNFR1 signaling axis with autolysosomal pathway and the accumulation of necrosome molecules in impaired lysosomes have been shown to lead to necroptotic neuronal death. This has been attributed to the terminal failure of the autophagic process, primarily due to lysosomal degradation dysfunction. Being the final and determining step of the autolysosomal pathway, lysosomes with sufficient acidification as maintained by functional vacuolar (H+)-ATPase (V-ATPase) are required to achieve complete autophagic degradation of toxic cellular components. In this study, we revealed that TNFR1 activated neuronal necroptosis correlates with autolysosomal dysfunction as characterized by downregulation of V-ATPase subunits and accumulation of autophagy receptor p62 in human postmortem AD brains. In cell culture, we showed for the first time that lysosomal acidification is only impaired in cells treated with TNF and not with other cytokines, contributing to inhibition of autophagic degradation in SH-SY5Y cells. We also illustrated that there is defective mitochondrial turnover, together with reduced mitochondrial functions and elevated reactive oxygen species, leading to neuronal death in SH-SY5Y cells. Importantly, we demonstrated that lysosome-targeting acidic nanoparticles restore lysosomal acidification, autophagic activity, and mitochondrial function, as well as rescue neuronal necroptosis in both TNF-treated SH-SY5Y cells and a mouse model of AD. This opens avenues for new therapeutic strategies to target lysosomal dysfunction to mitigate TNF-TNFR1 mediated neuronal death in AD.

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Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.01/F11

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: CRU 5024 - A01
IZKF Jochen-Kalenden Group N5

Title: Enteric Nervous System as a Hub for Immunometabolic Signalling in Mucosal Inflammation

Authors: *L. GONCALVES[1], B. WINNER[2], C. GÜNTHER[3], B. GHIROTTO[4];
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Abstract: Inflammatory Bowel Disease (IBD) encompasses Crohn's Disease (CD) and Ulcerative Colitis (UC), both of which are characterized by chronic intestinal inflammation. Emerging evidence suggests that alterations in gut metabolites are closely associated with CD, as disease-associated changes have been observed in patients with active disease. These metabolic shifts can influence immune signaling pathways, potentially contributing to disease progression. Additionally, increased intestinal permeability in CD may expose deeper layers of the gut, including the muscularis, where the myenteric plexus of the Enteric Nervous System (ENS) resides. Recent studies highlight a role for the ENS in modulating gut immune responses, though its contribution to IBD progression and the effects of altered gut metabolic environments on ENS function remain poorly understood. Firstly, we hypothesized that deep infiltration of these metabolites into the ENS compartment could trigger neural inflammation and enhance disease progression. To address this, our group employed a fully human ENS model in which we utilized an induced pluripotent stem cell (iPSC)-derived ENS differentiation system from healthy and IBD donors to examine the impact of the gut metabolome on ENS responses using high-throughput omics and functional analyses. We successfully differentiated enteric neurons and glia *in vitro*, as confirmed by the expression of *bona fide* markers of this compartment. Exposure to disease-associated gut metabolites led to transcriptomic changes that shifted the ENS toward a pro-inflammatory profile, accompanied by signs of metabolic stress, including ultrastructural mitochondrial changes and impaired respiratory function. Furthermore, we extended our investigation to understand how ENS-driven inflammation could impact the epithelial compartment. To this end, we employed the ENS-epithelial organoid co-culture model to study their communication upon metabolite treatment. Our findings show that the impact on the ENS compartment also shapes the function and viability of the epithelial stratum. Altogether, these findings establish a critical link between enteric neuronal immunity and environmental cues in IBD progression, offering valuable insights into potential therapeutic strategies and advancing our understanding of neural involvement in IBD immunopathology.

Disclosures: **L. Goncalves:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **B. Winner:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **C. Günther:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **B. Ghirotto:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.02/F12

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG grant 505539112
INST 90/1048-1 FUGG
BMBF – 031L0309A
ERC, DeepCell - 101054957

Title: Tnf-α Disrupts the malate-aspartate shuttle, linking mitochondrial dysfunction to enteric α-synuclein pathology in parkinson's disease and intestinal inflammation

Authors: ***B. GHIROTTO**, L. GONCALVES, C. JAMES, J. WINKLER, F. ZUNKE, B. WINNER;
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Abstract: Gastrointestinal (GI) dysfunction often precedes motor symptoms in Parkinson's disease (PD), implicating early involvement of the enteric nervous system (ENS) in disease pathogenesis. Alpha-synuclein (α-syn), the hallmark protein of PD, accumulates early in the ENS, and inflammation is believed to exacerbate this pathology. However, the mechanistic link between inflammatory signaling and metabolic vulnerability in enteric neural cells remains unresolved. Here, we identify TNF-α-induced disruption of the malate-aspartate shuttle (MAS) as a key mechanism linking inflammation to mitochondrial dysfunction and metabolic rewiring in the ENS. Using iPSC-derived enteric neural lineages (ENLs) from PD patients carrying α-syn triplications (SNCA 3x) and their isogenic controls, we show that TNF-α induces NAD^+ depletion, TCA cycle disruption, oxidative stress, and a compensatory shift toward glutamine oxidation in α-syn-vulnerable enteric neurons and glia. Multiomics profiling (scRNA-seq, proteomics, metabolomics) and functional assays revealed that TNF-α downregulates GOT1 (aspartate aminotransferase), alters substrate use, and increases mitochondrial α-syn accumulation in SNCA 3x ENLs. These effects were reversed by Chicago Sky Blue 6B, a glutamate metabolism inhibitor with known protective effects in PD models. To expand translational relevance, we analyzed public and in-house transcriptomic datasets from ulcerative colitis (UC) patients, a condition marked by chronic intestinal inflammation and high TNF-α. Across multiple transcriptomic datasets (bulk RNA-seq, microarray, scRNA-seq), MAS enzymes were consistently suppressed in inflamed gut, particularly within enteric neural clusters. GOT1 was suppressed in anti-TNF non-responders, with an inverse correlation to SNCA that

disappeared following successful infliximab therapy. UC biopsy staining confirmed these findings at the protein level. Together, our findings define MAS dysfunction as a conserved, inflammation-driven metabolic vulnerability in the human ENS. This work bridges synucleinopathies and intestinal inflammation, highlighting the power of iPSC-based modeling to uncover early disease mechanisms and therapeutic entry points in enteric neuropathology.

Disclosures: **B. Ghirotto:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **L. Goncalves:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **C. James:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **J. Winkler:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **F. Zunke:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen. **B. Winner:** A. Employment/Salary (full or part-time): Universitätsklinikum Erlangen.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.03/F13

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG KFO5024-505539112

Title: Sex-independent preservation of microglial homeostasis and adult neurogenesis in the hippocampal dentate gyrus during chronic colitis

Authors: ***R. K. MASANETZ**[1], J. WINKLER[1], C. GÜNTHER[2], P. SÜß[1];
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Abstract: Previous studies demonstrated both sex differences in basal adult hippocampal neurogenesis and differences in the formation of new neurons in the neurogenic niche of the hippocampal dentate gyrus upon inflammatory responses. A comprehensive understanding of the impact of chronic inflammation in peripheral organs such as the gut on the brain immune and neuronal compartments is of high clinical relevance. However, no studies have hitherto analyzed microglial activation and adult hippocampal neurogenesis in a genetic model of inflammatory bowel disease considering the impact of age and sex differences. To test the hypothesis that chronic gut inflammation induces microglial activation and proliferation, and thereby impairs adult neurogenesis in an age- and sex-dependent manner, we employed a highly specific genetically induced mouse model of humans Crohn‘s Disease. *Casp8*$^{\Delta IEC}$ mice lack *caspase 8* exclusively in intestinal epithelial cells (IECs) and spontaneously develop ileocolitis starting at an age of 12 weeks. Brain tissue of male and female *Casp8*^{fl} control mice and *Casp8*$^{\Delta IEC}$ mice aged 14- and 24-weeks was analyzed on transcriptional and histologic level to determine potential differences in cell number, proliferation, and activation of microglia and neuronal progenitor cells in the dentate gyrus. A similar density of Iba1^{+} microglia, and activation state based on morphology and expression of CD68, was observed regardless of age and sex. This was

further verified by similar mRNA expression of the *purinergic receptor P2Y12* and *apolipoprotein E*, markers for microglial homeostasis and activation, respectively. Comparing *Casp8*$^{\Delta IEC}$ to *Casp8*^{fl} mice, we detected a similar total number of Mcm2$^+$ proliferating cells in the subgranular zone of the dentate gyrus and DCX$^+$ immature neurons in the dentate gyrus as well as similar percentages of proliferating DCX$^+$Mcm2$^+$ cells of all DCX$^+$ cells. The resistance of adult hippocampal neurogenesis to chronic ileocolitis was further corroborated by similar expression of brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, and insulin-like growth factor 1 between groups independent of sex and age. Taken together, our findings indicate that microglial homeostasis and adult hippocampal neurogenesis are maintained during chronic ileocolitis independent of age and sex in *Casp8*$^{\Delta IEC}$ mice.

Disclosures: R.K. Masanetz: None. **J. Winkler:** None. **C. Günther:** None. **P. Süß:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.04/F14

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG CRU5024

Title: Olfactory bulb neurogenesis is resilient in a T cell-induced colitis model with severe inflammation of the colon

Authors: *M. ANDERT[1], T. KUNERT[2], K. HILDNER[2], A. HOFFMANN[3], J. WINKLER[2,1]; [1]Dept. of Mol. Neurol., Erlangen, Germany; [2]Univ. Hosp. Erlangen, Erlangen, Germany; [3]Dept. of Mol. Neurol., Keenan Res. Ctr. for Biomed. Science, St. Michael's Hospital, Toronto, ON, Canada;, Toronto, ON, Canada

Abstract: The gut-brain axis refers to a bidirectional interplay between both organs by which intestinal inflammation may propagate into the central nervous system. Intriguingly, patients with inflammatory bowel disease suffer from hyposmia and have an increased risk for developing Parkinson's disease, where hyposmia is known as an important premotor symptom. Olfaction is processed in the olfactory bulb (OB), one of the neurogenic niches in the adult mammalian brain. Given that neurogenesis is reduced in the hippocampus of colitis mice, this prompted us to examine whether OB neurogenesis may be affected by chronic intestinal inflammation. By utilizing a T cell transfer colitis model, we quantified neural precursor cells expressing doublecortin (DCX) as well as dopaminergic neurons, directly connected to sensory neurons of the OB. Additionally, since astrocytes are mediators of inflammation in the brain, we quantified cells expressing glial fibrillary acidic protein (GFAP) in distinct layers of the OB to evaluate their reactivity. DCX+ cells were observed along the rostral migratory stream (RMS), the route taken by neural precursor cells as they migrate from the subventricular zone (SVZ) to the OB. Reaching the OB, DCX+ neural precursor cells similarly integrate into distinct OB

layers independent of disease severity. The number and density of dopaminergic neurons predominantly localised in the ganglion cell layer of the OB did not correlate with the disease severity. Furthermore, the number of astrocytes was unaffected since GFAP+ reactive astrocytes were present independent of the level of gut inflammation. In contrast to the hippocampal neurogenesis, OB neurogenesis remains unaffected in a T cell transfer colitis model despite a severe chronic gut inflammation.

Disclosures: M. Andert: None. **T. Kunert:** None. **K. Hildner:** None. **A. Hoffmann:** None. **J. Winkler:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.05/F15

Topic: D.04. Brain Injury and Trauma

Support: NSF grant BRC-BIO 2313303
Hope College

Title: Role of inflammation in neurogenesis and repair in the olfactory system of adult zebrafish

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Abstract: Zebrafish (Danio rerio) are one of the few vertebrates that effectively repair brain lesions and constitutively generate new neurons throughout their lifespan, contrary to mammals, which have a limited capacity to repair neurons following damage. In particular, the olfactory system, which processes odors and is formed by the olfactory bulb (OB) and epithelium (OE), exhibits extensive neuroplasticity and repair mechanisms in response to damage.Neuroinflammation supports recovery and neurogenesis following damage in zebrafish, which lack classic stellate astrocytes and instead have radial-glia like astroglia and olfactory ensheathing cells in the olfactory system, which share functional similarities with mammalian astrocytes. Importantly, these cells have been described as neural stem cells (NSCs), supporting constitutive and damage-induced neurogenesis in the OB. The OE does not have residing astroglial cells, and contains two stem cell populations responsible for both constitutive turnover and regeneration of olfactory sensory neurons (OSNs), horizontal basal cells (HBCs) and globose basal cells (GBCs), respectively. However, the role of inflammation in the regeneration and repair of the OE is currently unexplored.In this work, we use a model of retrograde degeneration of the OE by excitotoxic lesions in the OB using quinolinic acid (QA) in adult zebrafish. We have previously established that these lesions cause neurodegeneration in the OE and olfactory functional deficits, and that neurogenesis supports recovery by 21 dpl. Here, we want to extend our findings and explore the role of inflammation in the recovery and

regeneration processes during early recovery at 1-, 6-, 24-, and 72- hr post lesion (hpl). We performed immunohistochemical examinations of the olfactory system and assessed markers of inflammation, cell proliferation, and neurogenesis: including GFAP (glial-fibrillary acidic protein; astroglial cells), Lcp1 (lymphocyte cytosolic protein 1; leukocytes), BrdU and EdU (proliferation markers), and HuC/D (mature neurons). To study the role of inflammation in cell proliferation dynamics, we used an anti-inflammatory drug, pranlukast, a cysteinyl leukotriene receptor antagonist. Our results provide a comprehensive time-course characterization of cell proliferation and inflammation dynamics in the olfactory system of zebrafish during the first 72 hpl. This work contributes to the understanding of the neurological mechanisms and associations between inflammation, neurogenesis, and neuronal recovery following damage in adult vertebrates.

Disclosures: **R. Post:** A. Employment/Salary (full or part-time): Hope College. **B. Didion:** A. Employment/Salary (full or part-time): Hope College. **O. Wiley:** A. Employment/Salary (full or part-time): Hope College. **N. Sanchez Gama:** A. Employment/Salary (full or part-time): Hope College. **L. Putt:** A. Employment/Salary (full or part-time): Hope College. **E. Calvo-Ochoa:** A. Employment/Salary (full or part-time): Hope College.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.06/F16

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Research Foundation of Korea (NRF) Grant 2023R1A2C1004955

Title: Oligodendrocyte precursor cell specific HMGB1 knockout reduces immune cell infiltration and demyelination in experimental autoimmune encephalomyelitis models

Authors: *G. KIM[1,2], S. LEE[1,2], D. LEE[1,2];
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Abstract: Infiltration and activation of peripheral immune cells are critical in the progression of multiple sclerosis and its experimental animal model, experimental autoimmune encephalomyelitis (EAE). This study investigates the role of high mobility group box 1 (HMGB1) in oligodendrocyte precursor cells (OPCs) in modulating pathogenic T cells infiltrating the central nervous system through the blood-brain barrier (BBB) by using OPC-specific HMGB1 knockout (KO) mice. We found that HMGB1 released from OPCs promotes BBB disruption, subsequently allowing increased immune cell infiltration. The migration of CD4+ T cells isolated from EAE-induced mice was enhanced when co-cultured with OPCs compared to oligodendrocytes (OLs). OPC-specific HMGB1 KO mice exhibited lower BBB

permeability and reduced immune cell infiltration into the CNS, leading to less damage to the myelin sheath and mitigated EAE progression. CD4+ T cell migration was also reduced when co-cultured with HMGB1 knock-out OPCs. Our findings reveal that HMGB1 secretion from OPCs is crucial for regulating immune cell infiltration and provides insights into the immunomodulatory function of OPCs in autoimmune diseases.

Disclosures: G. Kim: None. **S. Lee:** None. **D. Lee:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.07/G1

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Title: 2-cl-mgv1, a novel tspo ligand, promotes differentiation and survival of oligodendrocyte precursor cell line oln-93

Authors: *B. DAHLE[1,2];
[1]Neuropharm., Technion institute of technology, Turaan, Israel; [2]Med., technion institute of technology, Haifa, Israel

Abstract: Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by the death of oligodendrocytes and myelin loss. Current therapies primarily target the immune system; however, despite their effectiveness in mitigating disease severity, they do not repair immune-mediated damage to the myelin sheath. The translocator protein (TSPO) is an 18 kDa receptor located on the outer mitochondrial membrane, known to play a critical role in biological processes such as inflammation, cell differentiation, and survival. Oligodendrocyte precursor cells (OPCs) reside in the CNS and are recruited to lesion sites following demyelination, where they have the potential to differentiate and contribute to remyelination. 2-Cl-MGV1 is a novel TSPO ligand developed in our laboratory and has demonstrated anti-inflammatory effects in several in vivo and in vitro models. In this study, we investigated its potential role in promoting oligodendrocyte differentiation, survival, and remyelination. The rat oligodendrocyte precursor cell line OLN-93 was treated with various concentrations of 2-Cl-MGV1 for 7 days in Differentiation Medium. Differentiation was assessed via immunocytochemistry (ICC) using markers including MBP, CNPase, and GalC, along with morphological analyses such as cell body size, process complexity, cellular density, and myelin wrap formation, which were assessed through electron microscopy. Oligodendrocyte survival was evaluated using cytotoxicity and viability assays, including MTT. OLN-93 cells were incubated for 24 hours with conditioned media from three immune cell lines—RAW264.7, BV-2, and N9—with or without 2-Cl-MGV1. To assess myelin repair, OLN-93 cells were first differentiated using a standard differentiation cocktail for 7 days, followed by exposure to Lysophosphatidylcholine (LPC) and Lipopolysaccharide (LPS), with or without 2-Cl-MGV1, for an additional 48 hours. Myelin regeneration was assessed using ICC analysis of MBP and

CNPase expression. These findings suggest that 2-Cl-MGV1 enhances oligodendrocyte differentiation, promotes cell survival under inflammatory conditions, and supports remyelination following demyelinating insults. This highlights its potential as a therapeutic candidate for promoting myelin repair in demyelinating diseases such as Multiple Sclerosis.

Disclosures: B. Dahle: None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.08/G2

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: Taipei Hospital grant 11401-62-008

Title: Palmitic acid induces metabolic and mitochondrial alterations in hypothalamic POMC neurons

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[1]Natl. Chengchi Univ., Taipei City, Taiwan; [2]Inst. of Neurosci., Natl. Chengchi Univ., Taipei, Taiwan

Abstract: Obesity and related metabolic disorders are closely linked to hypothalamic inflammation, which develops in the early stages of a high-fat diet. The metabolic regulatory functions of the hypothalamus could be affected by the inflammatory environment and metabolites derived from the diet. Palmitic acid (PA), a predominant saturated fatty acid that is elevated in the brains of rodents fed a high-fat diet, has been shown to activate inflammatory signaling. Within the hypothalamus, pro-opiomelanocortin (POMC) neurons are key regulators of appetite and systemic energy balance. A series of in vitro and in vivo experiments were conducted to investigate the mechanisms underlying metabolic defects induced by hypothalamic inflammation. An immortalized hypothalamic POMC neuron cell line was used as a cell model and high-fat diet-fed mice were used as in vivo models to study the effects of PA on neuronal function. Prolonged PA incubation resulted in lipid droplet accumulation, increased ER stress, and ROS/RNS. However, these cellular stresses were insufficient to induce cell death. Cell viability assays, expression of pro-apoptotic genes, and TUNEL assays confirmed that apoptosis was not promoted. It is widely accepted that lipids are not the preferred energy source of neurons. However, PA accumulation increased aerobic respiration and induced metabolic alterations. Additionally, PA accumulation promoted mitochondrial biogenesis without interrupting neuronal activity. In summary, PA accumulation induces ER and oxidative stress but does not disrupt POMC neuronal activities. Metabolic defects induced by hypothalamic inflammation are possibly attributed to inflammation, rather than PA accumulation.

Disclosures: Y. Huang: None. **S. Chen:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.09/G3

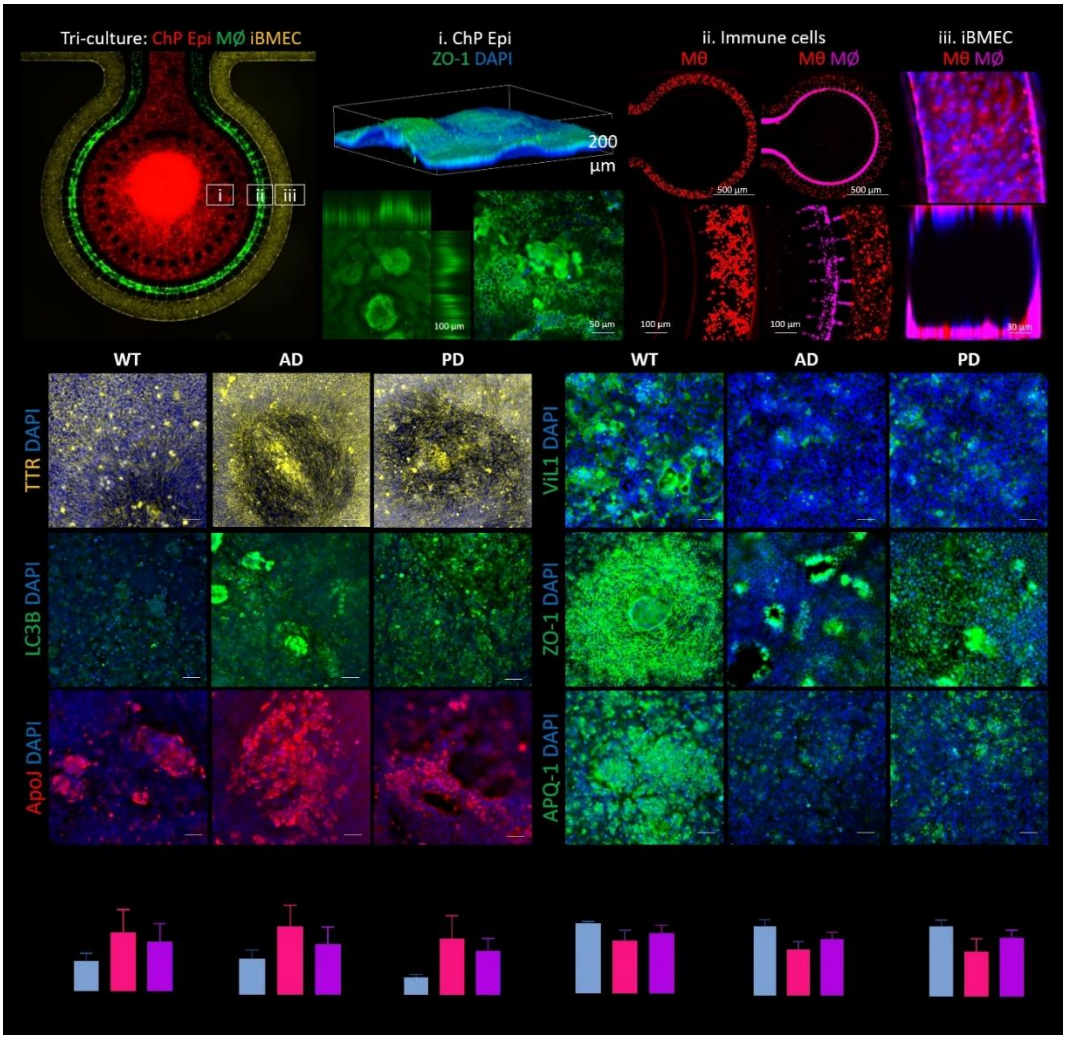
Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Research Foundation of Korea (NRF) grant
the Korea government (MSIT) (RS-2024-00336758)
Fourth Stage of Brain Korea 21 Project in Department of Intelligent Precision Healthcare, Sungkyunkwan University (SKKU) to H.C.

Title: Pathogenic Aggregates Induce MAO-B/NF-κB-Mediated Inflammatory Polarization in Humanized Villus Choroid Plexus-on-a-chip

Authors: *M. TRAN[1], H. CHO[2];
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Abstract: The choroid plexus (ChP), where the blood-cerebrospinal fluid barrier (BCB) resides, regulates immune cell trafficking and the clearance of neurotoxic substances. However, existing BCB models fail to capture key human features, including villus-like epithelial structures and dynamic immune interactions, limiting their relevance for studying cerebrospinal fluid (CSF) dysfunction. Here, we developed a humanized ChP-on-a-chip that integrates hESC-derived epithelial and endothelial cells with immune-competent stromal cells to replicate native epithelio-endothelial interactions. The model recapitulates key ChP physiology, including 3D crypt-villus epithelial architecture, intact tight junctions, and CSF-like fluid secretion. Exposure to neurodegeneration-associated DAMPs [$A\beta_{1-42}$, tau PFFs, and α-synuclein PFFs] led to upregulation of epithelial transthyretin receptor, reflecting enhanced uptake of these neurotoxic aggregates. Internalized aggregates impaired mitochondrial function through monoamine oxidase B activation, leading to excessive ROS signal translocation to the nucleus, ultimately disrupting protective pathways and initiating inflammatory polarization via NF-κB p65 activation. Converging proteostatic and oxidative stress impaired autophagic flux and lysosomal acidification, promoted Biondi tangle formation, disrupted epithelial barrier integrity, downregulated tight junction (AQP1, ZO-1, ViL-1), upregulated secretory stress markers (ApoJ, CLU), and drove progressive CSF barrier breakdown, further exacerbating neuroinflammatory responses. It was accompanied by robust recruitment of macrophages and peripheral monocytes into CSF while inducing pro-inflammatory (M1-like) phenotypes, and amplified cytokine-driven neuroinflammation, establishing a feed-forward loop of barrier disruption and inflammatory escalation. Our humanized BCB platform offers a physiologically relevant, fully human in vitro system for investigating ChP inflammation and epithelial injury in Alzheimer's and Parkinson’s disease.



Disclosures: M. Tran: None. **H. Cho:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.10/G4

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: AG082539
DA059310
MH106392
NS100624

Title: Peromyscus: establishing an innovative model for NeuroHIV

Authors: ***H. LI**[1], C. F. MACTUTUS[2], A. SIKIRZHYTSKAYA[1], V. SIKIRZHYTSKI[5], M. AKSENOVA[3], M. SHTUTMAN[4], R. M. BOOZE[6];
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Abstract: Traditional models of rodent HIV infection may not fully represent the genetic and phenotypic diversity characteristics in human populations. Peromyscus (aka deer mice) diverged over 25 million years ago from mice and rats and thus represents a distinct and ancient genetic lineage. In the current study, we developed a new, non-traditional model for NeuroHIV based on Peromyscus through EcoHIV infusion. First, in vitro, primary cortical cells treated with mScarlet-labeled EcoHIV for 48 hours, showed microglial EcoHIV expression. Second, in vivo, Peromyscus injected with EcoHIV-EGFP presented with a dramatic distribution of EcoHIV in the prefrontal cortex area after 2 weeks and combined with Iba1 staining further established microglial EcoHIV infection in Peromyscus. Next, we observed synaptodendritic alterations (pyramidal neurons from layers II-III of medial prefrontal cortex) through DiOlistic labeling technique. Significant alterations in neuronal dendritic arbors were found in the mPFC pyramidal neurons of Peromyscus following EcoHIV infection, relative to control. The complexity and extent of dendritic arbor alterations were indicated by a shift in dendrites closer to the cell body and loss of dendrites extending to more distal connections in the EcoHIV-infected Peromyscus. Additionally, using Cytokine/Chemokine 27-Plex Assay Array key biomarkers, IL-6, IL-10, TNFα and VEGF-A, provided comprehensive insights into inflammatory and immune responses after EcoHIV infusion in Peromyscus. Our data suggested that Peromyscus could be a valuable model for studying HIV-1 infection dynamics in producing NeuroHIV.

Disclosures: H. Li: None. **C.F. Mactutus:** None. **A. Sikirzhytskaya:** None. **V. Sikirzhytski:** None. **M. Aksenova:** None. **M. Shtutman:** None. **R.M. Booze:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

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Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: Korea Research Institute of Bioscience and Biotechnology (KRIBB) Research Initiative Program [KGM4562532]
National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-RS-2023-00229062)
Electronics and Telecommunications Research Institute (ETRI) grant funded by the Korean government [24YB1200]

Title: Biocompatibility assessment following neural electrode implantation in non-human primates

Authors: ***J. WON**[1], M. KIM[2], J. PARK[3], K. LIM[2], C.-Y. JEON[4], Y. LEE[3];
[1]Korea Res. Inst. of Biosci. & Biotech., Cheongju-si, Korea, Republic of; [3]Natl. Primate Res. Ctr., [2]Korea Res. Inst. of Biosci. and Biotech., Cheongju, Korea, Republic of; [4]Korea Res. Inst. of Biosci. and Biotech., Cheonju, Korea, Republic of

Abstract: Background and Objective: Objective. Subchronic implantation of neural electrodes in deep brain structures such as the subthalamic nucleus (STN) is essential for the advancement of long-term neuroprosthetic and neuromodulatory therapies. While the clinical efficacy of deep brain stimulation (DBS) has been widely demonstrated, there remains a paucity of preclinical data on the biocompatibility and functional safety of chronically implanted electrodes, particularly in non-human primates (NHPs). This study aimed to evaluate the tissue response and behavioral outcomes following subchronic implantation of custom microelectrodes targeting the STN in cynomolgus monkeys. Methods: Probe-type microelectrodes were stereotactically implanted into the subthalamic nucleus (STN) of adult cynomolgus monkeys (*Macaca fascicularis*) and maintained in situ for four weeks. Postoperative recovery and general health status were monitored daily. Locomotor performance was continuously evaluated using quantitative gait analysis, including parameters such as step length, symmetry, and limb coordination. At the study endpoint, brain tissue surrounding the electrode sites was collected for histological analysis. Results: During the 4-week implantation period, animals exhibited stable gait patterns without observable deficits in limb movement, stride length, or step symmetry, suggesting no significant motor impairment associated with electrode placement. Histological analysis revealed mild to moderate gliosis and microglial activation localized around the electrode tracks, while neuronal structures in adjacent regions remained intact. No evidence of widespread cortical or subcortical degeneration was found. The electrode-associated tissue responses were confined to the perielectrode region and demonstrated a spatially restricted glial reactivity profile, consistent with biocompatibility standards for subchronic neural implantation. Conclusion: Subchronic implantation of neural electrodes in the STN of cynomolgus monkeys was well-tolerated and did not affect gross motor function. The localized glial response supports the system's biocompatibility for use in preclinical neuromodulation research. These findings demonstrate the feasibility of long-term electrode-based interventions in NHPs and underscore the need for continued refinement of materials and implantation methods.

Disclosures: J. Won: None. **M. Kim:** None. **J. Park:** None. **K. Lim:** None. **C. Jeon:** None. **Y. Lee:** None.

Poster

PSTR069: Inflammatory Mechanisms and Immune Responses

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR069.12/G6

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: Wellcome Leap 1kD program
Eagles Autism Foundation
FAPESP 2024/02895-0
NGO "the tooth fairy project"
FAPESP 2024/21462-7
FAPESP 2024/10746-4
FAPESP 2024/22501-6

Title: Microbiome-driven epigenetic changes and their impact on neuronal functioning in the first years of life

Authors: *P. BELTRÃO-BRAGA[1], F. B. RUSSO[2], C. TADDEI[3], N. NASPOLINI[3];
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Abstract: Autism Spectrum Disorder (ASD) is a lifelong neurodevelopmental condition that affects communication, behavior, and social interaction on different levels. While its causes are not fully understood, both genetic and environmental factors are believed to contribute to this condition. The gut microbiome may influence brain development and function through the gut-brain axis, potentially contributing to the ASD phenotype. Additionally, scientific data suggest differences in the gut microbiota composition of individuals with ASD compared with those without ASD. These differences may affect brain function by altering gene expression and important signaling pathways, like the one involving glutamate, a neurotransmitter crucial for brain communication. Our work will investigate the relationship between the gut microbiome and ASD, focusing on how gut microbiota may influence brain function and how gut bacteria may impact brain development in individuals with ASD. To accomplish this, our group proposes investigating the microbiome composition and the products of bacterial metabolism in 100 Brazilian children with ASD, comparing the results with another group of children without ASD of the same age. Additionally, considering that microbiota bioproducts can influence gene expression, we will investigate the epigenetics of the participants, specifically, how genes can be turned on or off without altering the DNA sequence, helping to determine how DNA instructions are utilized. We participate in a multi-PI and Multi-Institute consortium (The First 1000 Days-1kD, LEAP). We followed and collected samples from 500 Brazilian neurotypical children aged 3 months to 3 years, analyzing their microbiome composition and methylome profile as they aged, and assessing their neurodevelopment using scales such as Bayles 3 and Bayles 8. Our data showed that gut microbiome composition was associated with PedBE-epigenetic clocks in three-month-old children. Clostridium perfringens and members of the Enterobacteriaceae family are positively correlated with PedBE scores in methylome analysis, as well as with the tryptophan pathway. Bacteroides spp is negatively correlated with cgs scores (inflammation). We will leverage the expertise gained in the 1KD consortium and the pipeline built from participating to extend our studies to individuals with ASD. Our data so far revealed that the gut microbiome of neurotypical Brazilian children differs from that of North America and Europe, which makes us believe that the microbiota of Brazilian children with ASD may also be different.

Disclosures: P. Beltrão-Braga: None. **F.B. Russo:** None. **C. Taddei:** None. **N. Naspolini:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.01/G7

Topic: D.05. Spinal Cord Injury and Plasticity

Support: JSPS KAKENHI (grant number 21H05049 to T.Y)

Title: A novel mechanism of spontaneous motor recovery following cervical dorsal root avulsion

Authors: *G. KANEMOTO[1,2], T. ITOKAZU[1], T. NAKANISHI[1], S. OKADA[2], T. YAMASHITA[1];
[1]Mol. Neurosci., [2]Orthopedic Surgery, Osaka Univ., Osaka, Japan

Abstract: Brachial plexus injury is one of the severe neurological injuries. Nerve root avulsion, which frequently occurs in brachial plexus injury, is a particularly serious type of nerve root damage, and a deeper understanding of its pathology is crucial for the development of novel therapeutic strategies. To address this issue, we developed a cervical dorsal root avulsion mouse model. Following C6 and C7 root avulsions, fine motor function was immediately disrupted, then spontaneously recovered in four weeks. In parallel with this, afferents from adjacent intact dorsal root ganglia exhibited compensatory sprouting toward injured spinal segments. In line with this, an increase in vGlut1 positive puncta per motor neuron was observed. Our finding suggests that plasticity of the afferent feedback circuit in adjacent dorsal root ganglia may contribute to the recovery of fine motor function following dorsal root injury. This neural recovery mechanism highlights a potential therapeutic avenue for promoting functional restoration after brachial plexus injury.

Disclosures: G. Kanemoto: None. **T. Itokazu:** None. **T. Nakanishi:** None. **S. Okada:** None. **T. Yamashita:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.02/G8

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation (32003B_208110)
Balgrist Foundation (2021-079)

Title: Monitoring reticulospinal plasticity and functional recovery after acute spinal cord injury

Authors: *A. EILFORT[1,2,3], L. C. NEUMANN[1,2,3], M. RASENACK[1], A. CURT[1,3], B. ZÖRNER[4], L. FILLI[1,3,5];
[1]Spinal Cord Injury Ctr., Balgrist Univ. Hosp., Zürich, Switzerland; [2]Dept. of Hlth. Sci. and Technol., ETH Zurich, Zurich, Switzerland; [3]Neurosci. Ctr. Zurich, Univ. of Zurich, Zurich, Switzerland; [4]Swiss Paraplegic Ctr. and Swiss Paraplegic Res., Nottwil, Switzerland; [5]Swiss Ctr. for Movement Analysis (SCMA), Balgrist Campus AG, Zurich, Switzerland

Abstract: Spinal cord injury (SCI) results in motor impairments below the level of the lesion. Individuals with incomplete SCI often show some degree of functional recovery, largely attributed to spontaneous neuroplasticity within descending motor pathways. Among these, the reticulospinal (RS) system —originating in the brainstem and involved in essential motor functions such as posture, locomotion, and reaching—has demonstrated a high capacity for neuroplastic adaptation in animal models. However, the role of the RS system in motor restoration after human SCI remains poorly understood.
This study investigates the involvement of the reticulospinal system in motor recovery following incomplete SCI. Ten individuals with acute SCI are assessed at 4, 12, and 24 weeks post-injury. At each time point, RS drive is evaluated using the StartReact paradigm, applied to the abductor digiti minimi, quadriceps vastus medialis, and tibialis anterior muscles. The StartReact effect - characterized by a reduction in reaction time when movement initiation is triggered by a loud vs. moderate acoustic stimulus - serves as an indirect measure of RS drive. Additionally, motor function is assessed using standardized clinical measures including the ISNCSCI motor score, GRASSP, maximal voluntary force, and gait assessments.
To date, five participants have completed assessments. Preliminary analyses reveal that changes in RS drive over time vary between participants: while some patients show enhanced RS drive over time, others exhibit a reduction. While group-averaged data demonstrate the presence of a StartReact effect, no consistent longitudinal increase in RS drive was observed in this preliminary analysis. In contrast, clinical measures indicate progressive functional improvements. These results indicate the complexity of the neural mechanisms driving functional recovery acutely post-injury.
The observed variability in RS plasticity during the subacute phase may be partially explained by the small sample size and the inherent heterogeneity of SCI (e.g., differences in lesion site and extent). A deeper understanding of brainstem involvement in early functional recovery can shed light into the mechanisms of neuroplasticity and inform targeted rehabilitation strategies.

Disclosures: A. Eilfort: None. **L.C. Neumann:** None. **M. Rasenack:** None. **A. Curt:** None. **B. Zörner:** None. **L. Filli:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.03/G9

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Swedish Research Council
Anna-Stina och John Mattsons Minnesstiftelse för sonen Johan
StratNeuro
Swedish Brain Foundation
Olle Engkvists Foundation
Karolinska Institutet

Title: Temporal coordination between cellular and extracellular adaptations supports neuronal functionality during regeneration of the adult zebrafish spinal cord

Authors: *L. LAFOUASSE, K. KOUTSOGIANNIS, Y.-W. DAI, L. DEL VECCHIO, A. PEDRONI, J. HABICHER, K. AMPATZIS;
Karolinska Institutet, Stockholm, Sweden

Abstract: The extracellular matrix (ECM) provides essential structural and biochemical support for neuronal function. Yet, its precise role in neuronal function following spinal cord injury and during regeneration remains poorly understood, particularly in the context of adult zebrafish, which exhibit remarkable regenerative capacity. Here, we investigate the intricate crosstalk between neuronal plasticity and ECM remodeling in response to spinal cord injury. Our findings reveal a precise and stereotypic temporal coordination between ECM restructuring and neuronal adaptations. Disrupting ECM remodeling through enzymatic digestion alters neuronal properties and the regenerative process, highlighting the critical role of ECM dynamics in shaping neuronal function. We propose a mechanistic framework in which glutamate signaling and ECM deposition cooperate to orchestrate spinal cord repair, ensuring functional recovery. These insights advance our understanding of the cellular and extracellular determinants of successful spinal cord regeneration and may inform the development of therapeutic interventions for spinal cord injury.

Disclosures: L. Lafouasse: None. **K. Koutsogiannis:** None. **Y. Dai:** None. **L. Del Vecchio:** None. **A. Pedroni:** None. **J. Habicher:** None. **K. Ampatzis:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.04/G10

Topic: D.05. Spinal Cord Injury and Plasticity

Title: Th role of spinal interneurons on breathing

Authors: *Y. Y. QU;
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Abstract: Normal 0 7.8 磅 0 2 false false false EN-US ZH-CN X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:普通表格; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0cm; line-height:115%; mso-pagination:widow-orphan; font-size:11.0pt; mso-bidi-font-size:12.0pt; font-family:等线; mso-ascii-font-family:等线; mso-ascii-theme-font:minor-latin; mso-fareast-font-family:等线; mso-fareast-theme-font:minor-fareast; mso-hansi-font-family:等线; mso-hansi-theme-font:minor-latin; mso-font-kerning:1.0pt; mso-ligatures:standardcontextual;} The role of spinal interneurons on breathing Breath originates from a central pattern generator (CPG) that produces rhythmic inspiration. The breathing CPG has at its core the preBotzinger Complex(preBotc),which relays inspiratory drive to the rostral ventral respiratory group(rVRG), thus directly innervating the phrenic motor neurons in cervical spinal cord. However, there are also date indicating that a ‘‘spinal respiratory rhythm generator’’ may could initiate rhythmic inspiratory activity independent of supraspinal input. In this study, we found hypercapnia recruiting spinal interneurons by cfos expression and single-unit recording. We further investigated the role of these spinal interneurons in physiology and pathology using the TRAP2 transgenic line. We found that inhibition of hypercapnia-TRAP cells impaired the respiratory response to hypercapnia, but not affect the maintenance of normal breath. After cervical spinal cord injury, we found that activation of hypercapnia-TRAP cells could restore the respiratory function. Then, we identified the hypercapnia-TRAP cells by snRNA-seq, and found that these cells are heterogeneous, including vglut2+, vgat+, chx10+ and so on. We further detected the effect of these cells on breathing, and observed that it’s vglut2+ cells that mainly contributed to the response to hypercapnia and function recovery after SCI.

Disclosures: Y.Y. Qu: None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.05/G11

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH/NINDS R01 NS121336
MU SCIDRP2024
MizzouForward Initiative

Title: Repeated autonomic dysreflexia does not aggravate renal dysfunction after spinal cord injury

Authors: R. BEN MUSA[1], Z. QI[2], C. FOOTE[3], ***S. HOU**[4];
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Abstract: Cardiovascular dysfunction is a common complication in individuals with spinal cord injury (SCI). One of the most serious consequences is autonomic dysreflexia, a life-threatening condition characterized by sudden and severe hypertension often accompanied by bradycardia, in response to somatic or visceral stimuli. While autonomic dysreflexia is known to adversely affect multiple organ systems, its impact on renal function remains poorly understood. Given the kidneys' sensitivity to blood pressure fluctuations, recurrent hypertensive episodes may compromise kidney perfusion and contribute to progressive renal dysfunction. To determine if repeated episodes of autonomic dysreflexia exacerbate renal function following SCI, adult female rats underwent a complete transection at the fourth thoracic (T4) spinal cord to eliminate supraspinal control. Two weeks later, a subset of SCI rats was subjected to colorectal distension (CRD) to induce autonomic dysreflexia twice daily for three weeks (SCI + CRD, $n = 8$). SCI rats without CRD ($n = 8$) and sham-injured rats (laminectomy only, $n = 9$) served as controls. Subsequently, renal function was assessed by noninvasive transcutaneous measurement of glomerular filtration rate (GFR) using fluorescein isothiocyanate (FITC)-sinistrin clearance. Blood and urine samples were collected to measure kidney injury markers. Furthermore, anesthetized rats underwent renal hemodynamic recordings to monitor or calculate parameters, including renal blood flow (RBF), renal vascular resistance (RVR), and mean arterial pressure (MAP). After sacrifice, the kidneys were harvested for histological examination. The results showed that GFR was significantly reduced in both SCI alone ($p = 0.0036$) and SCI + CRD ($p = 0.0003$) groups compared to shams. RVR was significantly elevated in both SCI alone ($p = 0.0317$) and SCI + CRD ($p = 0.0016$) groups. However, there was no difference in these parameters between SCI rats with and without CRD treatment. In both SCI groups, biomarkers of kidney injury displayed similar patterns. Urinary neutrophil gelatinase-associated lipocalin (NGAL) and protein levels were significantly increased 24 hours post-SCI, and plasma creatinine was elevated at one week. In addition, histological analysis with H&E staining did not detect obvious impairment in either the SCI + CRD or SCI alone group at 5 weeks. The results indicate that SCI induces acute kidney injury, while the impairment is attenuated over time. Repeated episodes of autonomic dysreflexia do not worsen renal function despite contributing to cardiovascular instability.

Disclosures: S. Hou: None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.06/G12

Topic: D.05. Spinal Cord Injury and Plasticity

Support: Japan Agency for Medical Research and Development (AMED), Grant Number 24bm1223008h0002

Title: Evaluation of epidural electrical stimulation therapy in a chronic severe spinal cord injury model

Authors: *Y. ICHIHARA[1], N. NAGOSHI[1], M. SHINOZAKI[2], H. OKANO[2], M. NAKAMURA[1];
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Abstract: [Background]
In chronic spinal cord injury (SCI), epidural electrical stimulation (EES) has been reported to induce locomotor movements by reactivating spinal circuits caudal to the lesion. Moreover, continued rehabilitation under EES (hereafter, EES rehabilitation) has been shown to enhance plasticity in spared pathways and partially improve voluntary lower limb control. However, the efficacy of EES in cases of severe SCI with minimal spared fibers remains poorly understood. In this study, we investigated the therapeutic effect of EES in a rat model of chronic severe SCI.
[Methods]
Female immunodeficient rats (8 weeks old) were used. A complete thoracic compression model was created by fully compressing the thoracic spinal cord using forceps. At 6 weeks post-injury, animals incapable of bipedal treadmill walking were selected for further study. We evaluated the optimal device design and stimulation parameters to induce bipedal stepping. EES rehabilitation was conducted for 7 weeks, and locomotor function as well as histological changes were compared between the control and EES rehabilitation groups.
[Results]
We developed a novel separate-type EES device that allows easy epidural placement and minimal mechanical burden on the spinal cord. Based on prior studies, we optimized stimulation settings and successfully induced bipedal stepping. After 7 weeks of EES rehabilitation, animals exhibited improved locomotor activity during stimulation (EES on). As in previous reports, a significant increase in c-Fos expression in Vsx2-positive neurons of the lumbar enlargement was observed. However, there was no significant difference in hindlimb motor function scores (BBB score) between groups in the absence of stimulation (EES off).
[Discussion]
EES reactivated neuronal activity in the lumbar enlargement, thereby improving stepping during stimulation. However, in cases of severe SCI with limited spared supraspinal connections, sustained EES rehabilitation alone was insufficient to restore descending communication, and thus voluntary hindlimb control in the EES-off condition did not improve.
[Conclusion]
In chronic severe SCI, EES treatment alone did not result in functional recovery of hindlimb motor ability.

Disclosures: Y. Ichihara: None. **N. Nagoshi:** None. **M. Shinozaki:** None. **H. Okano:** None. **M. Nakamura:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.07/G13

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH (HD36020(XYC)
NS22189(JRW)
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SCIRB C38338GG (JRW)
DOH-C38338GG(JRW)

Title: Long-term effects of sensorimotor cortex electrical stimulation on functional recovery in rats with incomplete spinal cord injury

Authors: *Y. CHEN[1], Y. WANG[2], X. Y. CHEN[3], J. S. CARP[4], B. HERRON[5], J. R. WOLPAW[6];
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Abstract: Operant up-conditioning of soleus H-reflex (SOL HR) improves locomotion in rats with incomplete spinal cord injury (iSCI) (J Neurosci 26:12537-12543, 2006). In healthy rats, weak electrical cortical stimulation (ECS) of sensorimotor cortex (SMC) increases SOL HR bilaterally (J Neurophysiol 108:2668-2678, 2012). We hypothesize that, in rats with asymmetric locomotion due to lateralized iSCI, ECS-induced bilateral HR increase will engage the impaired locomotor heksor to use the HR increase to restore locomotor symmetry (J Physiol 600: 3423-3452, 2022). The present study explores short and long-term ECS effects on HR and locomotion in rats with iSCI. Under anesthesia, Sprague-Dawley rats are implanted with EMG electrodes in right SOL, a nerve stimulating cuff on right posterior tibial nerve, and epidural stimulating electrodes over left SMC; and the lateral half of either right or left spinal cord is transected at T9. Each rat receives weak SMC ECS (25 <30-μA 40-ms bipolar pulses over 1s every 10s) for 30 d. ECS has no noticeable behavioral impact. HR data are collected throughout, and the rat practices treadmill locomotion 5d/wk. To explore long-term effects, some rats are studied 100 d after ECS stops. Horizontal ladder and treadmill walking are assessed before and after iSCI, after 30 d of ECS (acute effects) and 100 d later (long-term effects). We previously showed that 30 days of ECS produces an increase in SOL HR size like that with HR up-conditioning. In rats with lateralized iSCI, ECS plus treadmill training increases SOL EMG, improves step symmetry, and reduces foot drop. We have studied long-term effects in 4 rats to date. In these rats, reduced foot drop remained 100 d post-ECS. These early results indicate that ECS, like HR up-conditioning, increases HR size and improves walking in iSCI rats, and that benefits persist >3 months post-ECS. ECS may be an effective new therapy for locomotion deficits caused by iSCI, stroke, or other disorders.

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Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.08/G14

Topic: D.05. Spinal Cord Injury and Plasticity

Support: A Grant-in-Aid for Scientific Research on Innovative Areas "Hyper-adaptability" to TI (Project no. 19H05723)
A Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) to TI (KAKENHI (A) no. 19H01011 and (S) no. 22H04992)
A Grant-in-Aid for Scientific Research from MEXT to RY (KAKENHI (B) no. 21H02798)

Title: Large-scale plasticity associated with motor recovery from severe spinal cord injury in macaques

Authors: *R. YAMAGUCHI[1], T. KAWASAKI[1], Z. C. CHAO[2], S. UENO[1], M. MITSUHASHI[1], K. ISA[1], K. MATSUDA[1], T. TAKEI[3], H. ONOE[4], T. ISA[1];
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Abstract: Recent studies have shown that brain areas not typically involved in motor control contribute to motor recovery following neuronal injury. However, the underlying mechanisms remain unclear. In this study, we further investigated the motor networks involved in recovery following subhemisection spinal cord injury (SCI) at C4/C5 in macaques. We chronically implanted multi-channel ECoG electrodes over bilateral premotor (PM), primary motor (M1), and somatosensory (S1) cortices and monitored brain activity longitudinally. After the lesion, the monkeys were unable to perform a reach-and-grasp task. However, those that received cortical electrical stimulation (3 mA, 3 pulses at 20 Hz, 100 trains per site per week; 18 cortical sites per hemisphere, total 36 sites bilaterally) began to recover grasping ability between post-injury Weeks 3 and 4. Muscle twitches were first evoked from contralesional PM/M1 around Week 2 in proximal muscles, then gradually spread to distal muscles and were later evoked from ipsilesional PM/M1. These observations suggest large-scale plasticity in the motor networks during recovery. Next, we analyzed cortical activity and connectivity during movements. During reaching, activity in the ipsilesional PM/M1 increased in the β (13-30 Hz) band, and connectivity within the ipsilesional motor cortex, as assessed by Granger causality, was enhanced in the β band. During grasping, activity in the contralesional PM and M1 increased in the α (8-13 Hz) and high-γ (71-120 Hz) bands. Furthermore, connectivity from the ipsilesional PM to the contralesional PM/M1/S1 was enhanced in the α band during recovery. Finally, we investigated morphological changes in the corticospinal tract. In monkeys that exhibited global disinhibition, a greater number of labeled axons originating from the contralesional M1 were observed in bilateral spinal gray matter both rostral and caudal to the lesion site. In addition, more labeled

axons were distributed in laminae VIII and IX at the ipsilesional C6 level, where the hand motor nuclei exist. These findings suggest that bilateral motor cortices were involved in the recovery process from severe SCI and that different cortical networks contribute to recovery at distinct frequency bands.

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Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.09/G15

Topic: D.05. Spinal Cord Injury and Plasticity

Support: TCMF-MP 113–01–02
111–2314-B-303–028

Title: Effect of persistent epidural electrical stimulation on upper limb function in individuals with cervical spinal cord injury

Authors: *Y.-C. CHEN[1,2,3], S.-T. TSAI[3,4], C. WU[1,4];
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Abstract: **Motivation** Epidural electrical stimulation (EES) has significantly improved lower limb function in individuals with spinal cord injury (SCI). However, limited studies explore EES's potential to improve upper limb function for cervical-level SCI. The study investigates the effect of EES to improve the upper limb of individuals with cervical SCI. **Methods** Two participants with cervical level chronic SCI with American Spinal Injury Association Impairment Scale (AIS) A and B grade, respectively, and the mean age and injury duration were 40±21.2 yr. and 2.6±1.9 yr. The EES stimulator was implanted from C6 to T1 and deviated to the right side (preferred hand). In the three-month pre-intervention and post-intervention phases, two participants were assessed using the Action Research Arm Test (ARAT) to evaluate the performance of functional upper limbs. Additionally, surface electromyography (sEMG) was utilized to compare muscle activity. **Results and conclusion** The results revealed improvements in fine motor function. Specifically, the average ARAT grasp scores increased by 7 and 1.5 points on the right and left sides, respectively. Improvements were also observed in grip (2 points bilaterally) and pinch scores (2 points right, 1 point left). Additionally, the AIS motor score at the C8 level increased by one point. Enhanced muscle activity was observed in both wrist flexion and extension from sEMG. This study demonstrates that cervical EES can improve

fine motor functions, including grasp, grip, and pinch, in individuals with cervical SCI. These findings support the potential of EES as a promising intervention for upper limb motor recovery.

Disclosures: Y. Chen: None. **S. Tsai:** None. **C. Wu:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.10/G16

Topic: D.05. Spinal Cord Injury and Plasticity

Title: Functional recovery and predictive factors following spinal cord stimulation in chronic spinal cord injury

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[1]Hualien Tzu Chi Hosp., Hualien City, Taiwan; [2]Neurosurg., Hualien Tzu Chi Hosp., Hualien, Taiwan; [3]Hualien Tzu Chi Hosp., Hualien City, Hualien County, Taiwan; [4]Neurosurg., Hualien Tzu Chi Hospital, Buddhist Tzu Chi Med. Fndn., Hualien, Taiwan

Abstract: Background: Spinal cord stimulation (SCS) has demonstrated potential to enhance functional outcomes in individuals with chronic spinal cord injury (SCI). However, predictors of treatment responsiveness remain incompletely defined. Objective: To evaluate functional recovery outcomes following SCS and identify clinical and anatomical predictors associated with enhanced outcomes, thereby clarifying potential markers of neural plasticity. Methods: We retrospectively analyzed 19 individuals with chronic SCI who received epidural SCS. Motor and sensory scores were assessed at baseline, 3 months, and 12 months. Subgroup analyses were performed based on AIS grade, age, BMI, and the presence of tissue bridges on spinal imaging. Wilcoxon tests and generalized estimating equations (GEE) were used to assess within-group changes and interaction effects. Spearman's correlations were conducted to evaluate associations between continuous variables and functional gains. Results: Significant improvements were observed in motor ($p < 0.01$), sensory ($p < 0.01$), WISCI walking scores ($p < 0.05$), and VAS pain scores ($p < 0.01$) at both 6 and 12 months following SCS. Patients with AIS C injuries demonstrated greater functional gains than those with AIS A or B ($p < 0.05$). GEE analysis revealed significant interaction effects of SCS covert ratio ($p = 0.013$) and cumulative SCS treatment duration ($p = 0.045$), indicating that longer stimulation periods were associated with superior recovery outcomes. Tissue bridge presence showed a positive trend with improved sensory and walking recovery. Conclusion: SCS leads to clinically meaningful improvements in sensory, motor, pain, and walking ability outcomes in chronic SCI. AIS grade serves as a strong categorical predictor, while the SCS covert ratio and treatment timing independently contribute to recovery dynamics over time. These findings underscore the multifactorial nature of SCS responsiveness and support its role in long-term neurorehabilitation planning.

Disclosures: H. Huang: None. **S. Tsai:** None. **Y. Chen:** None. **C. Wu:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.11/H1

Topic: D.05. Spinal Cord Injury and Plasticity

Support: VA RR&D B7332R
NIH R01 NS104442
NIH R01 NS130033
NSERC

Title: Spinal cord injury-evoked hyperacute autonomic dysregulation in rhesus macaques - A distinct clinical syndrome with unique hemodynamic features

Authors: P. BARTMEYER[1], N. P. BISCOLA[2], R. MACON[3], R. WURR[3], E. SINOPOULOU[4], E. S. ROSENZWEIG[5], C. SPARREY[7], M. H. TUSZYNSKI[6], ***L. A. HAVTON**[8];
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Abstract: Spinal cord injury (SCI) results in varied neuromuscular impairment and autonomic dysregulation. Within hours or days after SCI, spinal shock may present with flaccid muscular paralysis and hypoactive reflexes, whereas acute neurogenic shock features suppressed autonomic responsiveness with hypotension, bradycardia, and reduced vascular perfusion. In contrast, delayed onset of autonomic dysreflexia after SCI includes evoked episodes of hypertension and reflexive bradycardia. However, information on immediate and hyperacute autonomic responses to SCI is sparse. We determined pulse rate (PR) and blood-pressure (BP) using continuous and non-invasive beat-to-beat recordings of vital signs in male rhesus macaques undergoing cervical spine surgery with a C5 laminectomy and a right-sided C6 hemi-contusion SCI. In addition, continuous PR, oxygen saturation (O2sat), and perfusion index (PI) recordings were collected intra-operatively by pulse-oximetry with sensors attached to hand and foot digits. Intraoperatively, BP, PR, PI, and O2sat recordings remained stable during the C5 laminectomy and pre-impact loading of the contusion probe against the exposed dura mater. However, a hemi-contusion SCI triggered an immediate and hyperacute increase in PR, detectable in all animals (n=9). The mean PR increased from 107.7±3.4 to 158.2±2.2 beats per minute ($p<0.001$), and the PR peak was reached at 16.1±1.8 seconds after the contusion SCI (n=9). The evoked PR increase was transient and showed a mean duration of 151.7±23.8 seconds (n=9). The rise in PR was followed by a transient decrease in SBP at 2.8±2.3 seconds after the onset of the PR rise (n=8). The mean SBP declined from 100.4±11.5 to 92.0±10.8 mmHg ($p<0.001$), reached its negative peak after 12.0±1.6 seconds, and had a duration of 73.2±13.2 seconds (n=8). Next, the SCI-evoked PR rise and SBP decline were followed by a transient increase in extremity PI, indicating increased peripheral vascular perfusion. In right hand digits,

the mean PI increased from 1.82±0.38 to 2.98±0.56 ($p<0.01$), peaked after 22.8±3.7 seconds, and had a duration of 217.6±32.1 seconds (n=9). In right foot digits, the mean PI increased from 1.69±0.36 to 2.53±0.49 ($p<0.001$), peaked after 27.4±3.8 seconds, and had a duration of 222.6±23.1 seconds (n=9). In conclusion, we show intra-operatively a previously not characterized hyperacute autonomic dysregulation syndrome, evoked by contusion SCI in non-human primates. Unique features of the hyperacute syndrome including tachycardia, reduced blood pressure, and increased perfusion of peripheral tissues.

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Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.12/H2

Topic: D.05. Spinal Cord Injury and Plasticity

Support: AMED JP25gm1510009
JSPS 23KJ1907

Title: Circulating low-density lipoprotein triggers pericyte-derived scarring following spinal cord injury

Authors: *Y. YONEZU[1,2], S. TANABE[1], H. MISAWA[2], R. MURAMATSU[1];
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Abstract: Spinal cord injury (SCI) causes irreversible neurological deficits, largely due to the failure of neuronal network regeneration. One major obstacle to regeneration is fibrotic scar formation. Fibrotic scar is formed by glutamate aspartate transporter (GLAST)-positive pericytes, following their proliferation. Therefore, elucidating the mechanisms underlying pericyte proliferation is expected to contribute to an understanding of fibrotic scar formation. However, the mechanisms that regulate pericyte proliferation remain unclear. In this study, we assessed the temporal dynamics of pericyte proliferation and blood extravasation following SCI. GLAST-positive pericytes proliferated during the acute phase when blood leakage was prominent, suggesting that circulating blood-derived factors may promote their proliferation. We searched for the factors containing in the blood that promote pericyte proliferation and found that low-density lipoprotein (LDL) promoted pericyte proliferation. LDL requires Apolipoprotein B-100 (ApoB-100) to interact with LDL receptor (LDLR). To ask the role of LDL *in vivo*, we conducted SCI in ApoB-100 deficient mice and found that ApoB-100 deficiency inhibited the proliferation of GLAST-positive pericytes and enhanced functional recovery after injury. We

also used pericyte-specific LDLR knockdown mice and found that the mice with silenced LDLR in pericytes showed lower pericyte proliferation and facilitated functional recovery compared with controls after injury. In human samples, we found an increase in the cultured pericyte proliferation by human LDL treatment. These findings provide the possibility that LDL is a novel trigger of fibrotic scarring after SCI and suggest that targeting ApoB-100 or its interaction with LDLR may represent a therapeutic strategy for improving neurological outcomes.

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Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.13/H3

Topic: D.05. Spinal Cord Injury and Plasticity

Support: A Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) to TI (KAKENHI (S) no. 22H04992)

Title: Motor recovery and corticospinal plasticity induced by repetitive transcranial magnetic stimulation after severe spinal cord injury in macaques

Authors: *L. ZHANG, M. MITSUHASHI, R. YAMAGUCHI, S. UENO, K. ISA, K. MATSUDA, T. ISA;
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Abstract: Spinal cord injury (SCI) at the cervical cord leads to paralysis of hands. Our laboratory demonstrated that while the spontaneous recovery after severe lesion (subhemisection) at the mid-cervical cord was slow and poor in nonhuman primates, a combination of rehabilitative training and electrical stimulation of the motor cortex through subdural electrodes could induce the plasticity of corticospinal tracts (CST) which bypass the lesioned area and promote recovery of motor function in the same SCI model (Yamaguchi et al. bioRxiv, 2023). To promote clinical applications, we aimed to develop a non-invasive stimulation approach by using repetitive transcranial magnetic stimulation (rTMS) to achieve similar recovery outcomes after subhemisection in macaques. We trained three monkeys (monkey-K, -D, and -M) to perform a reach-and-grasp task to assess finger dexterity. We conducted subhemisection at the border between C4 and C5 segments and performed rehabilitation combined with a novel rTMS protocol (3 pulses at 20 Hz every 2 s; 4500 pulses/session at 150 % rMT). The stimulation was applied to the motor cortex either of the contralateral or ipsilateral side to the SCI twice per week, respectively. For each monkey, we injected a viral tracer into the contralesional motor cortex to label the CST. Monkey-K was sacrificed 6 months after SCI, monkey-D and -M at 1.5 months. Monkey-A, as a control, underwent subhemisection and rehabilitation but without rTMS. We found that while the success

rate did not recover at all in control monkey (monkey-A), all the three monkeys with rTMS started recovery of grasping within weeks after the injury. The success ratio rapidly improved to over 90 % accompanied by a reduction in grasping time. Compared to the task performance in the first 1.5 months after SCI, monkey-K could pinch more precisely in the later months. We also recorded muscle twitches evoked by rTMS throughout the recovery process. Twitches in the distal muscles of the affected forelimb evoked by stimulation of the contralateral motor cortex were absent just after the injury but reappeared in parallel with the behavioral recovery. Moreover, we observed labeled corticospinal fibers in the C6 segment that crossed the midline to terminate in the ipsilesional gray matter. A greater number of these fibers were observed at 6 months (monkey-K) than at 1.5 months (monkey-M) after SCI. In conclusion, we successfully developed a non-invasive rTMS protocol that facilitates motor functional recovery after severe SCI in macaques. Our results suggest that plasticity in CST occurs in parallel with the behavioral recovery after SCI and further contributes to the recovery of precise finger movements.

Disclosures: L. Zhang: None. **M. Mitsuhashi:** None. **R. Yamaguchi:** None. **S. Ueno:** None. **K. Isa:** None. **K. Matsuda:** None. **T. Isa:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.15/H5

Topic: D.05. Spinal Cord Injury and Plasticity

Support: DOD # W81XWH-22-1-0998
NHLBI # 2R01HL117037-06A1
The Ellen R. and Melvin J. Gordon Center for the Cure and Treatment of Paralysis

Title: Neural oscillations during sleep in people with spinal cord injury (SCI): potential biomarkers of recovery and sleep-dependent plasticity

Authors: *L. M. MCCANE[1,2], C. TUTHILL[1], N. NIJK[1], M. SUKHOPLYASOVA[1], J. WALL[1], S. PATEL[1], N. PIAZZA[1], R. D. TRUMBOWER[2];
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Abstract: Over 18,000 individuals per year sustain spinal cord injury, which leads to loss of autonomic and voluntary function due to nervous system disruption. Improvements in recovery are slow and limited, especially after the first year (Burns et al., 2012). Sleep-dependent plasticity plays a crucial role in sensorimotor learning. Sleep disorders, particularly sleep-disordered breathing (SDB), affect > 80% of individuals with SCI (Sankari, et al., 2018) and may undermine the integrity of beneficial sleep processes. Thus, there is a critical need to understand the impact of SCI on sleep plasticity mechanisms and their effects on functional recovery.

Preclinical and clinical data reveal a model in which daytime cortical network activity is replayed during non-REM sleep through an interplay of subcortical and cortical oscillations that strengthen synaptic connections within the network (Klinzing et al., 2019; Rubin et al., 2022). Remarkably, sleep spindles (SSs)—brief 7- 15Hz bursts generated in the thalamus, which are essential for network replay, can be quantified from scalp EEG during polysomnography (PSG). Given that SS density (SS/min) increases with overnight sensorimotor gains (Baena et al., 2024) and decreases with limb disuse (Debarnot, et al., 2021), understanding their disruption in SCI could reveal new therapeutic targets for improving functional recovery. Adenosine, the primary endogenous substance that regulates sleep and wakefulness, undergoes homeostatic reduction during unfragmented sleep. SDB impairs adenosine clearance and is associated with SS deficits (Parker et al., 2022), possibly through elevated extracellular adenosine (Wall, 2022). We hypothesize that SCI-induced changes, such as SDB, result in diminished SS densities when compared to controls.To test our hypothesis, PSG data collected with a home-based recording device (A1s, Nox Medical, Reykjavik, Iceland) have been evaluated from 6 participants with SCI (5 M, age 18-29, BMI 20-33, 4 cervical, 2 thoracic, AIS grades A-C, 0.5-2.3 yrs since injury). Sleep stage scoring uses EEG from C3-M2 (200Hz, BP 0.3-35Hz). Calculation of SS density (13.5 Hz center) uses Morlet wavelet convolution as described in (Purcell et al., 2017). Compared to age-matched controls without neurological injury (Jonker et al., 2021), the participants with SCI had a 46% SS density reduction (range 0.04-3.6 SSs/min). Moderate SDB was noted in two individuals with SCI. The results of this ongoing study will fill a gap in knowledge about sleep-dependent plasticity in individuals chronic SCI, which is essential for recovery. They may reveal novel targets of sleep plasticity to enhance traditional therapies.

Disclosures: L.M. McCane: None. **C. Tuthill:** None. **N. Nijk:** None. **M. Sukhoplyasova:** None. **J. Wall:** None. **S. Patel:** None. **N. Piazza:** None. **R.D. Trumbower:** None.

Poster

PSTR070: Spinal Cord Injury: Plasticity and Recovery I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR070.16/H6

Topic: D.05. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01NS115025-01A1
Craig H. Neilsen Foundation 732676

Title: Optogenetic spinal stimulation to treat spinal cord injury

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Abstract: Spinal cord injury (SCI) causes significant functional deficits that greatly limit autonomy and quality-of-life. Unfortunately, there are no clinically available treatments that

restore functions back to pre-injury levels. However, for the past several years, our group has been evaluating the use of optogenetic spinal stimulation as a potential therapeutic following cervical SCI in rats with promising results. In the current study, neuron-specific optogenetic stimulation was delivered to female Long-Evans rats 1x/week beginning sub chronically at 4 weeks post-injury. Stimulation was positioned below the lesion, paired with a forelimb reaching task, and continued for a total of 6 weeks. Two weeks after the end of stimulation, rats were transcardially perfused, and tissue was processed to assess histological markers. Two additional groups of animals received epidural electrical stimulation below the lesion, or optogenetic stimulation *above* the lesion site. Our results show that optogenetic stimulation *below* the lesion led to significantly enhanced recovery on the forelimb reaching task, with rats performing at near pre-injury levels after 6 weeks of optogenetic stimulation, similar to our previous study. We also determined that optogenetically stimulated rats had significantly more axonal growth and synaptic density around motoneurons at the site of stimulation, with axonal growth exponentially increasing at regions closer to the stimulation site. These findings suggest that optogenetic stimulation may be both directing axonal growth toward the stimulation location, as well as promoting increased connectivity of newly formed circuitry. We also found enhanced NT-3 at the site of stimulation in optically stimulated rats. NT-3 has axon-guiding properties and may underlie the beacon-like effects on axonal growth seen with optogenetic stimulation. Notably, rats that received epidural electrical stimulation had less forelimb recovery than the optogenetically stimulated group. This suggests optogenetic stimulation may be a more robust neuromodulator that can promote better functional recovery after SCI. Additionally, optogenetic stimulation *above* the lesion did not promote enhanced forelimb recovery, indicating the importance of stimulation location caudal to the lesion site for promoting enhanced recovery. Overall, these results emphasize the promising therapeutic potential of optogenetic stimulation to treat SCI and begins to identify the underlying mechanisms of its success in hopes of future clinical translation.

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Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.01/H7

Topic: E.04. Interoception

Title: Paraventricular thalamic neurons encode peripheral signals through both humoral and vagal pathways

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[1]Dept Pharmacol, Grad Sch. Pharm, [2]Creative Interdisciplinary Res. Division, Frontier Res. Inst. for Interdisciplinary Scie, [3]Dept Neuropharmacol, Grad Sch. Med., Tohoku Univ., Sendai city, Miyagi, Japan

Abstract: Peripheral signals, such as inflammatory signals, are transmitted from the periphery to the brain through three major pathways: the humoral pathway, the vagal pathway, and the spinal pathway. However, it remains unclear which specific brain regions are activated by peripheral signals and through which pathways this activation is mediated. To address this, we conducted whole-brain mapping to identify brain regions activated by lipopolysaccharide (LPS)-induced peripheral inflammation. Using c-Fos immunostaining, we detected LPS-induced neuronal activation in 15 brain regions, including the paraventricular thalamus (PVT), medial prefrontal cortex, and nucleus of the solitary tract. Further, selective blockade of the humoral and vagal pathways significantly suppressed activation in 5 and 9 brain regions, respectively, while inhibition of the spinal pathway had no significant effect. These findings indicate that peripheral inflammatory signals are primarily transmitted to the brain via the humoral and vagal pathways. Notably, the PVT was the only brain region consistently activated regardless of pathway inhibition. To further examine how PVT neuronal activation depends on these two pathways, we performed single-unit recordings to observe neuronal firing patterns after LPS administration from anesthetized male mice. PVT neurons were classified into four types: those activated 0 to 20 minutes after LPS injection (20.7%), those activated 30 to 50 minutes after injection (27.4%), those activated during both periods (16.3%), and those activated during neither period(35.6%).Furthermore, blocking the humoral pathway with intracerebroventricular injection of alloxan suppressed neuronal firing in the 30- to 50-minute window, while blocking the vagal pathway reduced activity in the 0- to 20-minute window. These electrophysiological results confirm that PVT neurons receive signals through the humoral pathway and the vagal pathway. Our findings elucidate the transmission of peripheral signals to the brain and highlight the PVT as a central hub integrating peripheral signals conveyed through humoral and vagal pathways.

Disclosures: Z. Xie: None. **N. Kuga:** None. **T. Kayama:** None. **M. Yamakawa:** None. **T. Sasaki:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.02/H8

Topic: E.04. Interoception

Support: NIH Grant 1OT3OD025348-01
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NIH Grant 1OT3OD025349-01
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Title: Bridging Body and Brain: The SPARC Platform for Open, Collaborative Neuroscience

Authors: *J. BOLINE[1], B. DE BONO[2], P. HUNTER[3], N. KUSTER[4], E. NEUFELD[4], D. P. NICKERSON[3], M. E. MARTONE[5], J. B. WAGENAAR[6];
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Abstract: SPARC (RRID:SCR_017041) is a growing platform advancing scientific understanding and improving clinical outcomes through the integration of data, knowledge, modeling, and mapping of the peripheral nervous system (PNS), its CNS interactions, and related physiological processes. The SPARC Portal (https://sparc.science) is open-access, NIH compliant, HEAL-approved, and hosts high-quality FAIR datasets, computational and anatomical models spanning multiple species, organs, and modalities. Developed by the SPARC Data and Resource Center (DRC), the Portal provides essential infrastructure and interactive tools to investigate the pathways that bridge the body and brain.

The DRC is collaborating with SPARC Phase 2 teams to showcase their outcomes. The SPARC-V initiative (REVA and REVEAL) includes landmark structural and functional connectivity studies of the human vagus nerve. REVA is producing extensive vagus multimodal anatomical datasets. REVEAL, a clinical study, will offer new insights into the physiological and multi-organ effects of vagus nerve stimulation. The SPARC-O HORNET project is creating open-source biomedical devices. The Neuromod prize is accelerating development of targeted neuromodulation therapies. Outcomes from these projects will be available on the SPARC Portal.

With the unprecedented size, scale, and depth of the SPARC 2 datasets, new Portal features are being developed to visualize, explore, and reuse them. High-resolution imaging and segmentations of vagus nerves from REVA will be featured on a REVA Dashboard app that allows users to explore, visualize, and navigate data. Anatomical scaffolds (a geometric model providing a standard coordinate system) for the vagus nerve of each subject are being created for exploration and navigation. The Dashboard will also access information from a new Quantitative Database (QDB) that captures and serves values and measurements derived from these experiments. The QDB and REVA data can be leveraged from the o²S²PARC modeling platform for neural interface safety and efficacy assessment and optimization. Mechanical designs of HORNET devices will be featured with a new “dataset“ category - their CAD files can be opened in o²S²PARC for visualization and modeling.

Visit the SPARC Portal to learn about the Phase 2 projects and this new functionality. These enhancements reflect the DRC’s commitment to open science and amplifying the impact of research on the Portal. Investigators whose work aligns with SPARC’s mission are encouraged to publish through SPARC. SPARC provides an integrative platform to support our partners, showcase their work, and aid engagement with the broader community.

Disclosures: J. Boline: None. **B. de Bono:** None. **P. Hunter:** None. **N. Kuster:** None. **E. Neufeld:** None. **D.P. Nickerson:** None. **M.E. Martone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SciCrunch Inc.. **J.B. Wagenaar:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.03/H9

Topic: E.04. Interoception

Support: NIH Grant AI167933
NIH Grant AI176660

Title: Vagal Sensory Neurons Promote Anaphylactic Hypothermia

Authors: *K. SONG[1], H. IRIKI[2], R. SHIBUYA[1], B. S. KIM[1];
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Abstract: Food allergies, often seen in children and in patients with type 2 inflammatory disorders, can cause life-threatening systemic reactions known as anaphylaxis. A central mechanism in anaphylaxis is IgE-mediated activation of mast cells which can result in hypothermia and hypotension. Though mast cell-sensory nerve interactions have been elucidated in the pathophysiology of anaphylactic hypothermia, the specific neural populations involved remain unclear. Here, we explore neuroimmune mechanisms underlying food allergy-induced anaphylactic hypothermia. Mice were percutaneously sensitized to ovalbumin (OVA) and MC903, then orally challenged with OVA. Changes in rectal body temperature upon OVA challenge demonstrated that anaphylactic hypothermia is mast cell-dependent, and gastric mast cells were shown to accumulate in proximity to sensory nerves. Compartment-specific resiniferatoxin (RTX)-mediated ablation of TRPV1+ neurons in dorsal root ganglia and vagal ganglia revealed that vagal sensory neurons promote anaphylactic hypothermia. This phenotype was attenuated by subdiaphragmatic vagotomy as well as by chemical and surgical ablation of the gastric branch of the vagus nerve. Further, chemogenetic activation of vagal sensory neurons sufficiently induced hypothermia in non-sensitized mice. These findings highlight a novel role of vagal sensory neurons in food allergy-mediated anaphylaxis.

Disclosures: K. Song: None. **H. Iriki:** None. **R. Shibuya:** None. **B.S. Kim:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Allen Discovery Center, Doris Duke Charitable Foundation, NIAMS Grant AR070116, NIAMS Grant AR077007, NIAID Grant AI167933, NIAID Grant AI176660. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ABRAX Japan, Alys Pharmaceuticals, Attovia Therapeutics, Locus Biosciences, Neurommune Therapeutics, Recens Medical, Triveni Bio, Patent for the use of JAK1 inhibitors for chronic pruritus. F. Consulting Fees (e.g., advisory boards); ABRAX Japan, AbbVie, Amgen, Attovia Therapeutics. Other; Clexio Biosciences, Eli Lilly and Company, Escient Pharmaceuticals, Evommune, Galderma, LEO Pharma, Micreos, Novartis, Pfizer, Recens Medical, Regeneron Pharmaceuticals, Sanofi, Septerna, Triveni Bio, Triveni Therapeutics.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.04/H10

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: Stanford Bioengineering

Title: Genetic and cell type-specific indications of inflammatory bowel disease in the central and enteric nervous system

Authors: *L. LIMA[1], L. DUNCAN[1], T. P. COLEMAN[2];
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Abstract: The central (brain) and enteric (gut) nervous systems are deeply interconnected via the gut-brain axis, with each influencing the other's function. As a result, dysfunction in one can trigger a cycle of dysregulation in both. A growing body of research suggests a mechanistic linkage between dysfunction of the gut and brain. Studies have shown that mental health and psychological stress exacerbate GI flares of IBD, while both inflammation and permeability of the gut are implicated in the pathogenesis of neurodegenerative diseases. In this study, we attempt to bridge genomic analyses and cell-type specific transcriptomics in an effort to shed light on the role that IBD may play in brain function. Specifically, IBD disease risk genes from Genome Wide Association Studies were correlated with gut and brain tissue-specific expression from human single-cell RNA sequencing samples. We employed linear regression to model each cell type; this technique was adapted from a recent publication that explored brain cell types implicated in Schizophrenia (Duncan et al, Nature Neuroscience 2025). The regression coefficient was determined by the correlation of high-risk genes with high gene expression in healthy tissue samples; this is under the assumption that high expression indicates an important function, and in a diseased state, these genes are downregulated, thus contributing to pathogenesis. Preliminary analysis of IBD risk gene expression in central nervous system (CNS) cell types identified IBD associations of microglia and T cells in the brainstem and spinal cord. Within these cells, two genes stood out as being both highly expressed and significantly disease-risk associated: Runx3 and CCL5. Runx3 is a key immune regulator, whose dysregulation in the brainstem could send inflammatory signals to the gut, contributing to IBD. This dysregulation, commonly driven by stress, here shows the critical influence of brain health and stress on the gut. CCL5, a proinflammatory chemokine, may be dysregulated by IBD's gut inflammation relay to the brainstem via afferent vagal fibers. This altered CCL5 expression recruits immune cells and may contribute to neuroinflammation. Although these genes have been studied for their role in gut inflammation, these effects within the brainstem, implicating the CNS as an upstream contributor to IBD and IBD to neuroinflammation, has not been studied to this mechanistic extent in humans.

Disclosures: L. Lima: None. **L. Duncan:** None. **T.P. Coleman:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.05/H11

Topic: E.04. Interoception

Support: W81XWH-19-1-0802
Danone of North America Fellowship
UCR Mini-Grant
NIH MARC Summer Pre-Trainee Program

Title: Do Cholecystokinin Receptor Containing Vagal Afferent Neurons Mediate Gut-Brain Inflammatory Signals?

Authors: *E. KOZLOVA[1], A. LAM[1], S. ALAM[1], J. SHUM[1], M. DENYS[1], C. V. BERDASCO[2], G. DE LARTIGUE[3], M. C. CURRAS-COLLAZO[1];
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Abstract: Inflammatory responses are associated with chronic diseases and persistent fatigue and may contribute significantly to Gulf War Illness (GWI). The gut-brain axis is emerging as a conduit of cross-talk between central and peripheral inflammatory responses. Recent work has identified an important role for vagal afferent neurons (VANs) containing CALCA and TRPA1 receptors that regulate pro-inflammatory and anti-inflammatory responses, respectively. However, the contribution of cholecystokinin (CCK) receptor-containing VANs to gut-brain immune signaling is unknown. CCKRs are G-protein-coupled receptors that are activated by the satiety-inducing gut peptide CCK and are ubiquitously expressed in VANS, whose cell bodies are located in vagal nodose ganglia (NDG). We hypothesized that selective ablation of CCKR-containing VANs in NDG would dampen the inflammatory response after LPS challenge. C57BL/6N male mice underwent bilateral vagal deafferentation via injection of the neurotoxin, CCK-saporin (CCK-SAP) (500nL/NDG; 250ng/uL). Controls received a non-targeted peptide (SAP). Six days post surgery, mice were treated with LPS (0.1 mg/kg, ip) or saline. At sacrifice, 2h later, mice were perfused and plasma, brains and nodose ganglia collected. Plasma was processed for Interleukins (IL)-6, IL-1B and IL-10 via ELISA, nodose ganglia for *Cckar* and Toll-like receptor 4 (*Tlr4*) and brains for c-FOS immunofluorescence. RNAscope of *Cckar* mRNA expression confirmed deafferentation of CCKR-containing VANs. Cell counts of *Cckar* in VANs were markedly lower (63%) in CCK-SAP vs Blank-SAP ($p<0.0001$, n=10-12/group). Cell counts measured in sham controls receiving Blank-SAP were no different than those from non-operative controls (ns, n=5-10/group). The CCK-SAP method abolished a significant decrease in food consumption in response to ip CCK8 (4ug/kg) (n=7; $p<0.05$-0.0001). Mean normalized values of LPS-stimulated IL-6 expressed as a percent of corresponding saline control was significantly lower (51%) for CCK-SAP vs Blank-SAP ($p<0.05$, n=5-9/group). There were no changes in plasma IL-1B and IL-10 in CCK-SAP vs Blank-SAP. The percent increase in counts of c-FOS-positive cells in solitary tract nucleus (NTS) in response to LPS stimulation was significantly lower (29%) in the CCK-SAP vs Blank-SAP group ($p<0.01$, n=4-5/group)

indicating an interruption of gut-brain signals. *Cckar*-expressing VANs in NDG also expressed *Tlr4*, indicating a potential pathway for gut inflammatory signal reaching brainstem. Targeting gut-brain inflammatory signals via CCKR VANs opens a window into novel strategies for treatment of disorders with deregulated immune responses.

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Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.06/H12

Topic: E.04. Interoception

Support: Start-up funds and departmental funding

Title: Simultaneous Recording for Renal and Splenic Nerve Activity Reveals Coordinated Activity during Hypertension

Authors: *Z. YOUSUF[1], H. M. LIBREROS JIMENEZ[3], M. I. ROMEROORTEGA[2]; [2]Biomed. Engin., [1]Univ. of Arizona, Tucson, AZ; [3]Inst. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico

Abstract: Introduction. Hypertension is a major global condition affecting more than 1.3 billion people worldwide and a major cause of stroke. Chronic high blood pressure produces tissue damage and inflammation in critical organs such as the kidney, heart and brain. A neuroimmune response is initiated by increased blood pressure, stimulating T-cell activation in the spleen, which then migrate to affected organs to ameliorate damage. This suggests possible coordinated activity between these organs. We recently reported the application of sensitive, flexible platinized graphene electrodes (sutrodes) to record spontaneous and evoked electrophysiological activity in cervical vagus nerve (cVN), right renal nerve (RN) and splenic nerve (SN) terminal branches. We observed an increase in splenic neural spike frequency evoked by hypertension, presumably mediated by an efferent pathway, but whether it is coordinated with efferent renal nerve activity is unknown. We hypothesized that renal and splenic nerve activity is coordinated to reduce blood pressure. **Materials and Methods.** Sixteen male rats were divided into 2 groups: normotensive Wistar-Kyoto (WKY, n=8) and spontaneously hypertensive rats (SHR, n=8). Simultaneous recordings of sympathetic nerve activity were performed in cVN, RN, and terminal branches of SN. Flexible biocompatible platinized graphene fiber electrodes (sutrodes) were surgically implanted on each target nerve. Neural activity was recorded during baseline and pharmacologically induced modulation of arterial pressure to assess differential autonomic responses between groups. Neural activity was recorded using Omniplex Data Acquisition System at 40KHz sampling rate, high-pass filtered (4-pole Bessel) at 300 Hz and analyzed with Offline Sorter and NeuroExplorer software (Plexon, Inc). **Results.** The electrode

system enabled stable high-resolution recordings, supporting exploration of sympathetic crosstalk between renal and splenic innervation under hypertensive and normotensive conditions. We observed sequential changes in neural activity, with an initial decrease in cVN spiking, followed by tonic RN activity increase, then burst activation in SN branches 1 and 3. Power spectral analysis and statistical evaluation will be presented. We also found evidence that this coordination is mediated by neurons in the nodose ganglia. **Conclusion.** Our preliminary data indicates spleen and kidney function in response to hypertension is coordinated partly at the nodose ganglia. This can assist in improving neuromodulation therapies for drug-resistant hypertension.

Disclosures: Z. Yousuf: None. **H.M. Libreros Jimenez:** None. **M.I. RomeroOrtega:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.07/H13

Topic: E.04. Interoception

Support: Warren Alpert Grant
NIH R34
Simons
Burroughs Wellcome Fund
Branco Weiss Grant
Additional Ventures Grant

Title: Receptive fields of gut mechanics in the nodose ganglion

Authors: *A. ARAUJO[1], S. MELNYCHUCK[1], L. HERNANDEZ-NUNEZ[1,2];
[1]Harvard Univ., Cambridge, MA; [2]Dept. of Biol. and Wu Tsai Neurosciences Inst., Stanford Univ., Palo Alto, CA

Abstract: Vagal sensory neurons (VSNs) provide the primary viscerosensory input to the brain. However, their activity is rarely studied in unanesthetized, intact animals due to the need for invasive preparations that typically require anesthesia. Here, we leverage the optical accessibility of larval zebrafish to quantify gut movements while imaging neural activity across the entire population of VSNs in intact animals. We employ machine learning techniques to derive low-dimensional representations of gut movements and then construct encoding models that describe the dependency of vagal sensory neuron activity on these low-dimensional representations of gut mechanics. Based on these encoding models, we formulate functional hypotheses and then test them using optical manipulations of the gut. Our results reveal which features of gut movement are communicated to the brain through the vagus nerve and the role of these representations for feedback control of gut physiology.

Disclosures: **A. Araujo:** A. Employment/Salary (full or part-time): Harvard University. **S. Melnychuck:** A. Employment/Salary (full or part-time): Harvard University. **L. Hernandez-Nunez:** A. Employment/Salary (full or part-time): Harvard University.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.08/H14

Topic: E.04. Interoception

Support: Warren Alpert Grant
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Branco Weiss Grant
Additional Ventures Grant

Title: Encoding of multiorgan mechanics in vagal sensory neurons

Authors: ***T. YAN**[1], J. AVRAMI[1], A. ARAUJO[1], L. HERNANDEZ-NUNEZ[1,2];
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Abstract: Vagal sensory neurons (VSNs) provide the primary viscerosensory input to the brain. These inputs are essential for the body to regulate its internal environment to maintain homeostasis. However, their activity is rarely studied in unanesthetized, behaving animals due to the need for invasive preparations that typically require anesthesia. In this project, we take advantage of the optical accessibility of larval zebrafish to simultaneously quantify heart and gut mechanics while imaging neural activity across the entire population of VSNs in live, behaving animals. Extracting from these recordings, we generate an encoding map of multiorgan mechanical signals in the vagal system. Based on these maps, we formulate functional hypotheses and test them using optogenetics to selectively activate subsets of VSNs. Our results reveal spatial patterns of encoding and demonstrate how specific VSN subpopulations contribute to the neural feedback control of cardiac and gut physiology.

Disclosures: **T. Yan:** None. **J. Avrami:** None. **A. Araujo:** None. **L. Hernandez-Nunez:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.09/H15

Topic: E.04. Interoception

Support: Warren Alpert Grant
NIH R34
Simons
Burroughs Wellcome Fund
Branco Weiss Grant
Additional Ventures Grant

Title: Neural regulation of visceral adaptation to temperature change in larval zebrafish

Authors: *C. LEE[1], L. HERNANDEZ-NUNEZ[2];
[2]Dept. of Mol. and Cell. Biol., [1]Harvard Univ., Cambridge, MA

Abstract: Temperature is a critical environmental variable for aquatic poikilothermic animals such as fish, not only because it directly affects cellular physiology, but also because it alters dissolved oxygen levels in water. To survive, fish must adapt their visceral physiology to maintain homeostasis in response to fluctuating environmental conditions. Here, we leverage the optical and genetic accessibility of larval zebrafish to investigate how warming and cooling drive changes in cardiovascular physiology. We then examine the role of autonomic sensory circuits in orchestrating the necessary organ-level adaptations during thermal stress. Our findings highlight both the capabilities and constraints of physiological plasticity in fish exposed to temperature fluctuations, offering a framework for studying how developing fish may be challenged by ongoing climate change.

Disclosures: C. Lee: None. **L. Hernandez-Nunez:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.10/H16

Topic: E.04. Interoception

Support: JSPSKAKENHI 23K18356
JSPSKAKENHI 25K02975
LOTTE Research Promotion Grant 2025A0011

Title: Involvement of TRPV1 receptor in the initiation of swallows evoked by heat stimulation in anesthetized rats

Authors: *T. TSUJIMURA[1], C.-R. PAN[4], H. HAMASHIMA[4], Y. TSUTSUI[2], M. YOSHIHARA[5], J. MAGARA[4], K. OKAMOTO[6], M. INOUE[3];
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Abstract: Introduction: Thermosensitive transient receptor potential (TRP) channels are known as one of the targeted receptors for swallowing impairment. However, how thermal stimulation directly modulates the initiation of swallowing remains unclear. Here, we investigated the effect of laryngeal thermal simulation on the initiation of swallows. Methods: Experiments were carried out using fifty-nine urethane-anesthetized Sprague-Dawley male rats. A swallow was identified by digastric and thyrohyoid electromyographic bursts. Initially, we evaluated the effect of 3-sec thermal stimulation (10℃, 20℃ and 50℃) on the initiation of swallows using a peltier-controlled temperature delivering device placed on the vocal folds. Next, we investigated the effect of laryngeal application of 1 mM capsazepine, 1mM SB366791 (both of TRPV1 antagonists) and combination of 6 mM QX-314 and 10 μM capsaicin (QX/Cap, blockade of capsaicin-sensitive neurons) on 50℃- and 60℃-evoked swallows. We also investigated the effect of 10 μM capsaicin (TRPV1 agonist) and 1mM 2APB (TRPV2 agonist) on the initiation of swallows. Subsequently, we evaluated the effect of 10℃ and 20℃ laryngeal stimulation on the initiation of swallows evoked by electrical stimulation to pharyngeal mucosa (PhM). Additionally, we conducted immunohistochemical staining to identify TRPV1 expression in the laryngeal mucosa and vagal ganglia. Results: 50℃ stimulation evoked a number of swallows, while 10℃ and 20℃ stimulation failed to evoke a swallow. Both capsazepine and SB366791 significantly decreased the number of 50℃-evoked swallows. Although QX/Cap abolished 50℃-evoked swallows, many swallows were evoked by 60℃ stimulation after QX/Cap treatment. Capsaicin evoked many swallows, while 2APB evoked few swallows. The stimulus threshold for PhM-evoked swallows was significantly increased during both 10℃ and 20℃ laryngeal stimulation. TRPV1 immunoreactivity was observed in the laryngeal mucosa and in several nodose and jugular ganglion cells. Conclusions: We suggest that laryngeal heat stimulation evokes the swallowing reflex through activation of TRPV1, which is expressed in the laryngeal mucosa and vagal ganglia. On the other hand, laryngeal cold and cool stimulation might inhibit the initiation of swallowing at the central region.

Disclosures: T. Tsujimura: None. **C. Pan:** None. **H. Hamashima:** None. **Y. Tsutsui:** None. **M. Yoshihara:** None. **J. Magara:** None. **K. Okamoto:** None. **M. Inoue:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.11/I1

Topic: E.04. Interoception

Support: NIH DP1 AT009497
Howard Hughes Medical Institute

Title: Understanding vagal neurons in the heart.

Authors: *Z. LIU, S. LU, S. LIBERLES;
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Abstract: The heart is richly innervated by vagal and spinal sensory neurons, autonomic motor neurons, and intrinsic neurons of the cardiac nervous system. These cardiac neurons enable precise moment-to-moment regulation of heart rate and blood pressure, protect the heart from injury, mediate cardiovascular adjustments during posture changes and exercise, influence mood, and contribute to autonomic states such as the 'fight-or-flight' response. Recent single-cell profiling studies have revealed a striking diversity of sensory, motor, and intrinsic neurons that innervate the heart. However, the functions of many of these heart-innervating neurons remain poorly understood. In particular, how sensory information from the heart is represented in the brain—and how central neural circuits generate motor commands to the heart—remains largely unknown. While PIEZO2-expressing neurons in arteries are known to detect blood pressure changes and mediate the baroreceptor reflex, many other cardiovascular sensory terminals remain enigmatic. Here, we investigate the mechanisms and pathways underlying heart-brain communication. Our primary goals are to characterize the diverse sensory neurons that innervate the heart and nearby vessels and to understand the autonomic reflexes they engage. This work lays a critical foundation for the development of novel therapies targeting the heart-brain axis.

Disclosures: Z. Liu: None. **S. Lu:** None. **S. Liberles:** F. Consulting Fees (e.g., advisory boards); Nilo Therapeutics.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.12/I2

Topic: E.04. Interoception

Support: U01NS113868
U01DK116311
OT2OD023854
1R01DK141890

Title: Role of Piezo ion channels in stretch-evoked activation of vagal afferents that innervate the esophagus.

Authors: *S. NAIR, M. PATIL;
Univ. of South Florida, Tampa, FL

Abstract: The esophagus receives dual innervation of extrinsic afferent fibers from both the dorsal root ganglia as well as the vagal ganglia. The nodose ganglia give rise to low threshold mechanoreceptors (LTMRs) that innervate the esophagus which sense esophageal stretch. This

mechanotransduction has been shown to play a critical role in primary peristalsis and is essential for secondary peristalsis. PIEZO2 is the bona fide mechanosensitive ion channel in mammals. Physiological functions that involve mechanosensation such as sensing lung inflation and aortic blood pressure is under the control of PIEZO ion channels expressed in a subset of vagal afferents. We hypothesize that PIEZO2 is an essential mechanotransducer of esophageal stretch in nodose esophageal LTMRs. We used a GCaMP6s multiphoton imaging approach to measure mechanosensitive afferent activity in nodose neurons following esophageal distension using an ex-vivo intact vagal ganglia-esophageal preparation connected by the vagus nerve. Preliminary data in PIRT-GCaMP6s mice showed a profound increase in Ca2+ influx in a subset of nodose neurons in response to mechanical distention of the esophagus. Pharmacological inhibition of PIEZOs using GsMTx4 caused a ~9%, ~18% and ~30% reduction in fluorescence intensities at 5,10 and 30mmHg pressure, respectively. We generated a nodose neuron-specific PIEZO2 knockout mouse with GCaMP6s expression by crossing $P2X_2$-Cre:GCaMP6s mice ($P2X_2$ is expressed in all nodose neurons) with $PIEZO2^{fl/fl}$ mice ($P2X_2$:$GCaMP6s^{PIEZO2}$ KO). Knockout was confirmed by qPCR (~4.3 fold reduction in PIEZO2 transcripts) as well as intraperitoneal FM1-43 injection, which showed a significant reduction (54% in controls to 14% in KO mice) in the number of dye-positive neurons in the nodose ganglia (n=3 mice). In comparison to control $P2X_2$-Cre:GCaMP6s, these $P2X_2$:$GCaMP6s^{PIEZO2}$ KO mice showed a significant reduction of 50%, 20%, 38% in fluorescence intensities at 5, 10, and 30 mmHg pressure, respectively ($p<0.05$, 3 mice each group). Lastly, the addition of GsMTx4 to the esophagi of $P2X_2$:$GCaMP6s^{PIEZO2}$ KO mice further reduced distension-evoked responses at all three pressures, suggesting an additional role for PIEZO1. In conclusion, our data indicates that PIEZO2 plays an important role in vagal esophageal mechanosensation with PIEZO1 also making a significant contribution.

Disclosures: S. Nair: None. **M. Patil:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.13/I3

Topic: E.04. Interoception

Support: R01HL152219
OT2OD023854
U01NS113868
T32HL160529
R01DK141890

Title: Anatomical and functional mapping of vagal nociceptive sensory nerve subsets innervating the mouse lower airways by intersectional genetics

Authors: *M. PATIL[1], S.-H. KIM[2], K. LURYE[3], T. DARCEY[3], S. HADLEY[4];
[1]MPP, [2]Dept. of Mol. Pharmacol. and Physiol., [3]Univ. of South Florida, Tampa, FL; [4]Univ. of South Florida, TAMPA, FL

Abstract: Most vagal sensory afferents innervating the lower airways are activated by noxious stimuli including irritants (e.g. TRPV1 agonist capsaicin) and inflammatory mediators, causing nociceptive cardiorespiratory reflexes (e.g. cough, bronchospasm, changes in respiratory drive and heart rate). Vagal ganglia are comprised of embryologically distinct nodose and jugular neurons, but little is known of their specific contribution to nociceptive reflexes. Using a novel $TRPV1^{Flp}$ mouse in combination with $P2X2^{Cre}$, $Tac1^{Cre}$, intersectional reporter mice and AAV we mapped and modulated distinct nociceptive afferents. $TRPV1^{+}P2X2^{+}$ neurons were found exclusively in the nodose ganglion and were activated by αβmATP and capsaicin but rarely expressed Tac1. $TRPV1^{+}P2X2^{+}$ fibers innervated the lungs (many projected into the alveoli) but not the trachea. Centrally they innervated the nucleus tractus solitarius (nTS). >90% of $TRPV1^{+}Tac1^{+}$ neurons were found in the jugular ganglion and were activated by capsaicin but not αβmATP. $TRPV1^{+}Tac1^{+}$ fibers innervated the lungs (although none projected into the alveoli) and the trachea submucosa. They terminated solely in the paratrigeminal complex (Pa5). Many $TRPV1^{-}Tac1^{+}$ neurons were found in both nodose and jugular ganglia that innervated the trachea and large pulmonary airways. These projected to both nTS and Pa5. Using intersectional chemogenetics we selectively stimulated lower airway afferent subsets using intravenous injections of clozapine-N-oxide (CNO). Activation of $TRPV1^{+}$, $TRPV1^{+}P2X2^{+}$ or $TRPV1^{+}Tac1^{+}$ fibers evoked bradycardia and bradypnea. Activation of $Tac1^{+}$ fibers evoked tachycardia and tachypnea. Activation of vagal $TRPV1^{-}Tac1^{+}$ neurons only evoked tachycardia. These data show the distinct innervation patterns and reflex function of multiple nociceptive vagal afferent subsets.

Disclosures: M. Patil: None. **S. Kim:** None. **K. Lurye:** None. **T. Darcey:** None. **S. Hadley:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.14/I4

Topic: E.04. Interoception

Title: Neuroendocrine Cells Drive Airway Protective Reflexes

Authors: *L. SEEHOLZER[1], D. J. JULIUS[2];
[1]Neurobio., Stanford Univ., Stanford, CA; [2]Dept. of Physiol., Univ. California-San Francisco, San Francisco, CA

Abstract: Every breath exposes our airways to potential threats - like chemicals, gastric acid, food or liquids - which can damage the respiratory epithelium, induce inflammation and acutely

impair breathing. Our airways have multiple sensory systems that detect these external threats to trigger airway protective reflexes, like sneeze, cough, or swallowing. I found that rare epithelial cells in the trachea and larynx called neuroendocrine (NE) cells are sensitive to the commonly aspirated noxious stimuli, water and acid, and upon stimulation release ATP which activates purinoreceptive sensory nerve fibers to initiate swallowing and cough-like reflexes. Overall, my work uncovered striking molecular and biophysical diversity of NE cells across the airways and revealed molecular mechanisms by which these specialized cells serve as sentinels for activating a critical nociceptive circuit. My recent work investigates the functional importance of other molecularly distinct sensory epithelial cells.

Disclosures: L. Seeholzer: None. **D.J. Julius:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.15/I5

Topic: E.04. Interoception

Support: JSPS KAKENHI Grant 22KK0139

Title: Effect of potassium on swallowing reflex in anesthetized rats

Authors: N. PHETNIN[1], S. KAWADA[1], T. CHOTIRUNGSAN[2], Y. TSUTSUI[1], H. HAMASHIMA[1], M. ONO[1], J. MAGARA[1], T. TSUJIMURA[1], ***M. INOUE**[1];
[1]Niigata Univ., Niigata, Japan; [2]Dept. of Oral Diagnosis, Naresuan Univ. Fac. of Dent., Phitsanulok, Thailand

Abstract: Background and objective: The swallowing reflex can readily be induced by laryngeal stimulation. However, the mechanism of peripheral receptors involved in swallowing initiation is still unknown. In this study, we evaluated the effects of potassium ion containing solution on peripheral afferent responses and swallowing initiation in conscious and anesthetized rats. Furthermore, the possible receptors involved were explored. Methods: Experiments were performed on 7-9 w Sprague-Dawley male rats. First, the number of swallows was compared among 3-µl distilled water (DW), solutions containing potassium (KCl, KF, and K2SO4) and sodium chloride (NaCl) at several concentrations. Second, afferent discharges of superior laryngeal nerve (SLN) were recorded for 1 min after topical application of 3-µl DW, saline (160 mM NaCl) and KCl (160 mM) to the vocal folds. Third, licking behavior was compared among DW, saline and KCl (160 mM) in conscious animals. Fourth, the number of KCl evoked swallows was compared before and after topical application of KATP and Inward rectifier potassium (Kir) channel blockers. Finally, we investigated expression of Kir6.2 and Kir3.1 in nodose ganglion (NG) and vocal fold mucosa by immunohistochemical staining. Results: Topical application of KCl significantly facilitated the swallowing reflex; these facilitatory effects were more prominent than those of DWor NaCl; this phenomenon was dependent not on

the concentration of anions, but on that of potassium ions. The potassium ion-induced response in the SLN was the most prominent after treatment with KCl, especially at the early stage. In chronic rats, without differences in licking behaviour among DW, NaCl, and KCl, the intervals between swallows were the smallest during KCl-associated licking. Kir3.1 and Kir6.2 channel-positive cells were detected in the nodose ganglion and vocal folds; the rate of expression of these molecules in immunoreactive cells was relatively high: 74.1% for Kir3.1 and 75.3% for Kir6.2. Kir3.1 and Kir6.2 blockers significantly decreased the number of KCl-induced swallows. Conclusion: Our findings suggest that Kir3.1 and Kir6.2 are involved in potassium ion-induced swallowing in rats.

Disclosures: N. Phetnin: None. **S. Kawada:** None. **T. Chotirungsan:** None. **Y. Tsutsui:** None. **H. Hamashima:** None. **M. Ono:** None. **J. Magara:** None. **T. Tsujimura:** None. **M. Inoue:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.16/I6

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DK103901
NIH Grant DK134773
NIH Grant DK141106
NIH Grant AR077183
NIH Grant AA027065

Title: Function of CGRPβ-expressing cholinergic IPANs in gut motility and inflammation

Authors: *Z. XIE, H. HU;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The enteric nervous system (ENS) is essential for gastrointestinal (GI) homeostasis, coordinating digestive functions, immune responses, and gut-brain communication. In Hirschsprung's disease, the absence of ENS neurons in the distal colon results in impaired motility and dysregulated intestinal inflammation. Despite the critical roles of the ENS, the mechanisms by which it regulates motility and immune responses remain poorly understood. In this study, we employ intersectional genetic approaches to identify intrinsic primary afferent neurons (IPANs) expressing calcitonin gene-related peptide β (CGRPβ) as critical regulators of GI motility. We demonstrate that CGRPβ-expressing IPANs are both necessary and sufficient to drive coordinated motility through cholinergic signaling within the ENS. Moreover, inhibition or ablation of CGRPβ-expressing IPANs disrupts normal GI motility and exacerbates GI inflammation, marked by increased recruitment of inflammatory monocytes, macrophages, and neutrophils. These findings delineate a dual role for CGRPβ-expressing IPANs in coordinating

GI motility and modulating immune responses via distinct molecular pathways. This work reveals the therapeutic potential of targeting CGRPβ-expressing IPANs to treat GI motility disorders and inflammation.<!--EndFragment-->

Disclosures: Z. Xie: None. **H. Hu:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.17/I7

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDCR R01DE031053

Title: Sting signaling in sensory neurons aids in host defense against Listeria monocytogenes

Authors: *B. DARWISH[1], G. BASSETT[1], A. ROBERTS[2], S. JAYACHANDRAN[3], A. MCGINNIS[1], A. ANDRIESSEN[4], C. DONNELLY[5];
[1]Duke Univ., Durham, NC; [2]Duke Univ., DURHAM, NC; [3]Ctr. for Translational Pain Med., Duke Univ., Cary, NC; [4]Anesthesiol., [5]Dept. of Anesthesiol., Duke Univ., Durham, NC

Abstract: Listeria monocytogenes (L.m.) is a bacterial pathogen responsible for the foodborne illness listeriosis and is among the most common bacterial causes of meningoencephalitis among newborns and immuno-compromised individuals. Once L.m. crosses into the central nervous system (CNS), lethality exceeds 50%, even with aggressive medical treatment. The mechanisms that opportunistic bacterial pathogens such as L.m. utilize to gain access to the CNS are still poorly understood. A suspected method for *L.m.* to enter the CNS is through peripheral sensory neurons which serve as a conduit. We have previously demonstrated that the innate immune regulator STING is highly expressed by pain-sensing nociceptors in peripheral sensory ganglia and in spinal microglia. In this study, we sought to determine how STING signaling in peripheral sensory neurons impacts CNS invasion and survival outcomes in a mouse model of L.m.-induced infection. To this end, we challenged mice with various titers of *L.m.* (10^5 to 10^6 CFU/ml, *i.p.*) and developed a scoring system to evaluate symptomatic progression, morbidity, and mortality. We analyzed invasion of *L.m.* into neural tissues of the brain, spinal cord and peripheral sensory ganglia. We performed L.m. challenge experiments in wildtype mice, mice lacking STING globally (gKO/ $Sting1^{gt/gt}$), or mice lacking STING selectively within peripheral sensory neurons (cKO/ $STING^{fx/fx}$; $Na_v1.8$-Cre). We also evaluated morbidity and mortality outcomes in mice following intrathecal administration of diABZi, a small molecule STING activator. Notably, we observed significantly poorer morbidity and mortality outcomes after in vivo L.m. challenge in both Sting-gKO and Sting-cKO when compared to their control littermates. Compared to vehicle-treated controls, male mice administered intrathecal diABZi exhibited significantly improved survival; protective effects were not observed in female mice. Collectively, these data suggest that STING signaling in peripheral sensory neurons confers protective immunity against

L.m., potentially offering a therapeutic strategy to mitigate morbidity and mortality arising from neuroinvasive pathogens.

Disclosures: B. Darwish: None. **G. Bassett:** None. **A. Roberts:** None. **S. Jayachandran:** None. **A. McGinnis:** None. **A. Andriessen:** None. **C. Donnelly:** None.

Poster

PSTR071: Peripheral Mechanisms: Vagal Afferents and Chemosensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR071.18/I8

Topic: B.09. Glial Mechanisms

Title: Lpa-induced activation of satellite glia cells and its modulation by pipe-791, a novel lpa_1 antagonist, in an in vitro pain model

Authors: *K. I. LORRAIN;
Biol., CONTINEUM therapeutics, San Diego, CA

Abstract: The dorsal root ganglia (DRG), part of the peripheral nervous system (PNS), contains astrocyte-like satellite glial cells (SGCs) that closely envelop neuronal cell bodies. SGCs are implicated in nerve regeneration and pain signaling and express LPA1 (lysophosphatidic acid receptor 1). Given the established link between LPA signaling in the dorsal root ganglia (DRG) and neuropathic pain, we investigated the response of satellite glial cells (SGCs) to LPA stimulation, both in the presence and absence of PIPE-791, a novel selective LPA_1 receptor antagonist. Previous studies have shown that lysophosphatidic acid (LPA) levels increase following injury, leading to the activation of satellite glial cells (SGCs), which in turn amplify pain signaling pathways—an effect primarily mediated by the LPA_1 receptor. We generated primary rat SGC cultures and confirmed both Glutamine Synthetase (GS) and FABP7 (fatty acid binding protein 7) expression. Previous data has shown that LPA induces rapid calcium release via intracellular stores; similarly, when we measured calcium mobilization after LPA, we observed rapid induction. This was completely inhibited in the presence of PIPE-791, confirming not only that LPA induces calcium release, but that release is mediated by LPA_1. To assess SGC activation, cells were treated with LPA for 3 hours, which led to an upregulation of glial fibrillary acidic protein (GFAP), a well-established marker of SGC activation. Interestingly, we also observed upregulation of FABP7 with LPA as well. Notably, PIPE-791 significantly reduced the expression of GFAP and FABP7, reinforcing the role of LPA signaling in neuropathic pain and demonstrating that LPA_1 antagonism can effectively attenuate glial activation. Collectively, these findings suggest that PIPE-791 may alleviate pain *in vivo* by modulating LPA-induced activation of satellite glial cells (SGCs).

Disclosures: K.I. Lorrain: None.

Poster

PSTR072: Visual Cortex: Circuits

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR072.01/I9

Topic: E.06. Vision

Support: NIH Grant EY030550

Title: Synaptic mechanisms governing the integration of corticocortical and higher-order thalamocortical pathways across sensory processing hierarchies

Authors: M. HALLER, *G. T. NESKE;
State Univ. of New York, Univ. at Buffalo, Buffalo, NY

Abstract: Context-dependent sensory processing requires the selective routing of information across multiple regions of the neocortex. Functionally distinct cortical regions communicate information in both feedforward and feedback directions not only by direct synaptic connections (corticocortical (CC) pathways), but also indirectly via thalamocortical (TC) connections arising from higher-order thalamic nuclei. For instance, primary visual cortex (V1) and secondary visual cortex (V2) are bidirectionally connected not only by CC axons originating from each area, but also by TC axons originating in the pulvinar nucleus (the higher-order nucleus of the visual system). Thus, the processing and flow of information across the cortex likely depends upon the precise synaptic interplay between these two classes of afferents. Unlike for primary sensory cortex and first-order thalamus, however, the synaptic properties and connectivity of long-range CC pathways and their higher-order TC counterparts are not well understood. Here, we combine transsynaptic tracing techniques with intersectional genetics in the mouse visual thalamocortical system to uncover the distinct properties of CC and higher-order TC projections at several hierarchically defined processing levels. Using G-deleted rabies tracing, we find that higher-order TC neurons at the earliest visual processing stages primarily receive corticothalamic input from a single source. Using pathway-specific synaptic bouton labeling, we find distinct laminar termination patterns for feedforward CC and higher-order TC inputs originating from the same cortical source and terminating in the same cortical target. In contrast, the laminar termination patterns of the feedback projections are largely complementary. By expressing ChR2 in pathway-specific CC and higher-order TC axons and performing whole-cell patch clamp recordings from cortical neurons combined with optogenetic stimulation, we find that feedforward CC and higher-order TC synapses from V1 to V2 are dynamically distinct, with divergent AMPA and NMDA components. Using dual-color optogenetic activation of CC and higher-order TC afferents, we are currently uncovering the connectional logic underlying the integration of CC and higher-order TC synaptic inputs across distinct visual cortices. Overall, our current results indicate that CC and higher-order TC pathways, despite originating from the same cortical source, operate in distinct dynamical and functional regimes. These results may provide a synaptic basis for the distinct roles of these pathways during active visual processing.

Disclosures: M. Haller: None. **G.T. Neske:** None.

Poster

PSTR072: Visual Cortex: Circuits

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR072.02/I10

Topic: E.06. Vision

Support: Max Planck Society

Title: Representational stability despite sustained circuit disinhibition in mouse visual cortex

Authors: *U. LEWIN[1], J. BAUER[1,2], T. BONHOEFFER[1], M. HUEBENER[1];
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Abstract: Neuronal representations in cortex are not fixed but gradually change over time—a phenomenon known as *representational drift*. While several studies have characterized the phenomenology of drift, the mechanisms that limit it, enabling stable feature representation, remain unclear. Orientation preference in mouse visual cortex is a well-suited model system for investigating the principles that govern stable feature representations. It offers both a comparably detailed understanding of the underlying circuitry and shows moderate but reliable representational drift.

Inhibitory interneurons, particularly parvalbumin-positive (PV) cells, are strong candidates for limiting drift of orientation preference, given their central role in controlling pyramidal neuron excitability and modulating plasticity across development. PV interneuron activity sharpens orientation tuning, and since broader tuning curves have been linked to greater drift in a computational model, we hypothesized that PV interneuron activity stabilizes orientation representations by limiting the tuning width of excitatory neurons.

To test this, we chemogenetically reduced PV interneuron activity in the primary visual cortex of adult mice for one week, and performed repeated two-photon calcium imaging over weeks to track the orientation preference of excitatory neurons. As expected, inhibiting PV interneurons increased overall neuronal activity, broadened tuning curves, and elevated pairwise correlations. Surprisingly, however, these circuit-level changes had no effect on the magnitude of representational drift.

Thus, while PV interneurons in primary visual cortex alter the activity and tuning of excitatory neurons, they do not govern the long-term stability of preferred orientation. Given that the local circuit architecture of excitatory and inhibitory neurons is largely conserved across cortical areas, our results suggest that an inhibition-dependent increase in feature selectivity does not affect representational stability.

Disclosures: U. Lewin: None. **J. Bauer:** None. **T. Bonhoeffer:** None. **M. Huebener:** None.

Poster

PSTR072: Visual Cortex: Circuits

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Program #/Poster #: PSTR072.03/I11

Topic: E.06. Vision

Support: DFG Emmy Noether VE 938/2-1
DFG CRC-TRR:INST 39/1422-1

Title: Selective Inhibition of Principal Cells by Somatostatin Neurons Revealed by Foundational Modeling and Ultrafast Optogenetics

Authors: *D. ERIKSSON[1], C. RAMANATHAN[2], R. SHANKAR[4], J. VEIT[3];
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Abstract: Somatostatin-expressing (SST) neurons are differentially engaged by environmental, behavioral, and cognitive variables. However, measuring their causal impact on principal neurons in vivo remains challenging. Here, we combined wide-field ultrafast optogenetic suppression, electrophysiology, and foundational modeling in the visual cortex of the mouse to develop a generalizable approach for studying neuronal interactions.

To resolve the impact of a source (S) neuron onto a target (T) neuron using wide-field optogenetic suppression, we estimated their activity (S and T) immediately before every perturbation. The effect on the target neuron was quantified by its pre-suppression (T) and peri-suppression (B - background) activity. These three variables (S, B, and T) across multiple suppressions define a generalized impact function between neurons, with connectivity as a special case (Eriksson, 2017).

We expressed Jaws in V1 of SST-Cre mice and recorded activity with Neuropixels probes. To phototag SST neurons and extract the impact function, we delivered 20-ms wide-field light pulses. We optogenetically identified 87 putative SST neurons.

Wide-field illumination will typically affect more neurons than one can record from. To estimate how other, unrecorded, neurons respond to the visual stimulus we aggregated the stimulus response functions of all neurons across 16 sessions and 7 animals using a foundational model. Each neuron's identity was encoded as a 133-dimensional vector reflecting tuning to brain/state and spatio/temporal visual features. We reconstructed the activity of unrecorded SST neurons by inferring their pre-suppression activity (50 ms window) from the foundational model. This enabled computation of the impact function based on principal neuron activity changes within the first 8 ms post-suppression onset.

Our approach revealed significant, yet selective, inhibition from SST to principal neurons, with the inhibitory strength increasing with greater similarity between the identity vectors of SST and principal neurons. This selectivity may have implications for subtractive prediction-error coding and excitation-inhibition balance.

The millisecond-scale optogenetic suppression enhances interpretability by restricting indirect effects to the local microcircuit. Combined with large-scale electrophysiology and foundational modeling, this approach opens new avenues for probing causal interactions across heterogeneous neuronal populations during natural brain function.

Eriksson D., A Principle for Describing and Verifying Brain Mechanisms Using Ongoing Activity. Front. Neural Circuits, 2017

Disclosures: D. Eriksson: None. **C. Ramanathan:** None. **R. Shankar:** None. **J. Veit:** None.

Poster

PSTR072: Visual Cortex: Circuits

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Title: Layer 6 callosal projecting neurons provide bi-directional gain control between the monocular primary visual cortices

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Abstract: Interhemispheric communication between the two visual cortices relies on the corpus callosum, which is composed of axons from callosal projecting neurons (CPNs) that until now have largely been attributed to cells residing in cortical layers 2/3 and 5.
Here, we demonstrate that the monocular region of the primary visual cortex (VISp) in mice is anatomically and functionally interconnected primarily through a subset of excitatory Layer 6 (L6) cortico-cortical neurons representing a robust but non-topographically organized L6-L6 interhemispheric connection. Electrophysiological recordings in monocular VISp whilst presenting eye-specific visual stimulation restricted to the animal's monocular visual fields revealed short-latency (~ 20 ms) responses to contralateral, but also long-latency (~ 50 ms) responses to ipsilateral stimulation. Chemogenetic silencing of CPNs in one hemisphere led to increased spontaneous firing and contralateral- and ipsilateral-visually evoked activity across all layers in contralateral monocular VISp. Silencing CPNs was also found to unmask activity in neurons that were previously visually unresponsive. Despite observing such clear functional consequences of CPN silencing, both orientation tuning to drifting gratings and the ipsilateral responses to monocular visual field stimulation were however found to be unaffected. Overall, optogenetic activation of L6 CPNs was observed to have two distinct effects on neuronal firing in the contralateral monocular VISp: short-latency (5-10 ms) excitatory responses and delayed (20-100 ms) inhibition of ongoing activity. We find both in vivo and in vitro that the short-latency L6 spiking activity results from callosal-driven excitation of fast-spiking interneurons, which subsequently leads to suppression throughout the cortical column. Through this interhemispheric pathway, L6 CPNs were found to modulate cortical responses in the

contralateral hemisphere by regulating the gain without altering the tuning of individual neurons. These findings demonstrate that a novel L6-L6 circuit provides a locus for reciprocal columnar modulation of the sensitivity of so-called monocular VISp neurons to features of both the monocular and binocular visual world.

Disclosures: S. Weiler: None. **M. Vélez-Fort:** None. **T.W. Margrie:** None.

Poster

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Topic: E.06. Vision

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Title: Integrating feedforward and recurrent contributions to divisive normalization in mouse V1

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Abstract: Divisive normalization is a “canonical computation” common to sensory processing across multiple cortical areas, explaining the response properties of cortical neurons to multiple stimuli simultaneously presented in their receptive field. Phenomenological models of normalization successfully explain cross-orientation suppression in primary visual cortex (V1), but the underlying mechanisms within thalamocortical circuitry remain debated. Modeling work has proposed ed that intracortical inhibition could implement cross-orientation suppression, but direct evidence for response properties in inhibitory neurons consistent with this role has been limited. In contrast, alternative models suggest that feedforward circuits from LGN to V1 are sufficient to perform this computation with some support for this mechanism in data.
In this work, we record the responses of parvalbumin-expressing (PV) interneurons and excitatory neurons simultaneously in awake mouse V1 during the presentation of sine-wave gratings and plaids designed to evoke cross-orientation suppression. We use two-photon imaging with the fast calcium indicator GCaMP8s to record the responses of several hundred cells per mouse in the superficial layers of V1, imaging a 750x750um field of view at 30Hz and labeling PV interneurons with the constitutively expressed fluorescent protein tdTomato. Spike inference with the CASCADE algorithm allows us to extract neural responses with higher temporal precision than is possible from the raw fluorescence signals.
Our results suggest that local recurrent inhibition mediated by PV interneurons contributes to the emergence of divisive normalization in V1, in addition to the feedforward contribution previously shown. PV cells exhibit a transient reduction in suppression around stimulus onset, while suppression in excitatory neurons is anticorrelated with their population coupling—the

extent to which their activity tracks the broader network. Both observations are captured by the Allen Institute's V1 microcircuit model and by simplified stabilized supralinear network models, supporting a unified framework in which PV-mediated recurrent inhibition complements feedforward mechanisms to implement cross-orientation suppression. In addition, these network models suggest a relationship between normalization and E/I balance, where neurons with more tightly balanced excitatory and inhibitory inputs are more strongly suppressed, linking this canonical computation to theoretical work on the dynamical regime of cortex.

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Poster

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Topic: E.06. Vision

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Title: Perceptual deficits and impaired visual coding in excitatory and inhibitory neurons across multiple cortical areas in a mouse model of autism

Authors: *K. JACOBSON[1], B. HAIDER[2];
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Abstract: Impaired sensory processing is a core feature of autism spectrum disorder (ASD), involving both hyper- or hyposensitivity to sensory stimuli (Robertson and Baron-Cohen, 2017). Many studies have linked these sensory impairments to altered neural coding of sensory features in primary sensory cortex; however, higher order sensory areas are critical for sensory perception (Gilbert and Sigman, 2007), and these higher order areas have also been implicated in ASD (Schwarzkoft et al., 2014). It is not known if neural activity deficits in primary sensory areas impact sensory processing in downstream higher cortical areas, nor how these effects may relate to sensory impairments observed in ASD. We address this here by performing optically targeted high density neural recordings (Neuropixels) in primary visual cortex (V1) and multiple higher visual areas (HVAs) in a mouse model of ASD (CNTNAP2$^{-/-}$ knockout, KO) while they perform a psychometric visual detection task. Consistent with our previous studies (Del Rosario et al., 2021), our cohort of KO mice (n = 8) showed diminished perceptual performance versus wildtype controls (WT, n = 6), taking significantly longer to detect stimuli at all intensities (p=2e-5), and requiring more intense visual stimuli (higher contrast) than WT mice to reach the same performance level (p=7e-3). In those mice with subsequent days of neural recordings (n = 6 KO, n = 4 WT), we found altered excitatory and inhibitory neural activity and sensory coding across multiple cortical areas, with distinct profiles in each area, a topic of ongoing work. In general, neural sensitivity to stimulus contrast was reduced in putative excitatory (but not

putative inhibitory) neurons across V1 and all HVAs in KO mice (n=331 putative excitatory; n=274 putative inhibitory neurons; p = 0.02), mirroring reduced perceptual sensitivity to contrast in these same mice. Furthermore, neural response latencies showed significant delays across V1 and specific downstream HVAs in KO versus WT mice. These initial results suggest that 1) area-specific alterations to cortical excitation-inhibition in ASD models extend to higher sensory cortical areas, and 2) diverse, area specific deficits in neural coding and temporal processing of sensory information could contribute to perceptual impairments as relevant for autism.

Disclosures: K. Jacobson: None. **B. Haider:** None.

Poster

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Topic: E.06. Vision

Support: NIH Grant BRAIN Initiative NINDS NS132288
NIH Grant NEI EY036078

Title: Functional and cellular specificity of feedback from frontal to visual cortex in mice

Authors: *A. D. LIEN, B. HAIDER;
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Abstract: Top-down cognitive control of sensory processing involves frontal cortex feedback to sensory areas. A well-known example is when spatial attention improves perception of visual stimuli in a particular region of space. This is thought to rely on retinotopically-matched cortical projections from frontal to visual cortex. The precision of this control is constrained by the specificity of projection neurons and their targets in multiple visual cortical areas. However, the areal and retinotopic specificity of feedback projections from neurons with known visuospatial receptive fields (RFs) in frontal cortex has not been demonstrated. We recently identified a subpopulation of neurons in the mouse frontal cortex (M2 and anterior cingulate) with spatially localized RFs. Here we record from these frontal neurons, map their RFs, and use a novel antidromic “opto-tagging” approach to reveal the specificity of their axonal projections across retinotopic maps in V1 and higher visual areas (HVAs). We mapped axonal projection patterns by optogenetically evoking antidromic spikes from the axons of frontal cortex neurons labelled with channelrhodospin. We validated that these spikes are antidromic by showing 1) sub-millisecond jitter 2) failure when “colliding” with ongoing spontaneous spikes, a classically established metric 3) persistence during pharmacological block of synaptic transmission. By systematically surveying the likelihood of evoking antidromic spikes from retinotopically defined visual cortical areas (identified via intrinsic signal imaging), we reveal the functional axonal projection patterns of single neurons in frontal cortex with well-defined visual RF properties. 12% of frontal neurons (142/1194; 3 mice) were antidromically activated from visual

cortex, of which 32% had visual RFs. Projections of frontal neurons with RFs covered much of visual cortex including V1 and HVAs. Individual neurons had heterogeneous and specific projections including single- and multi-areal patterns. Previously we found that visually-evoked spiking of frontal neurons was strongly biased for stimuli in the binocular (central) region of space. Here we find that frontal neurons that project to visual cortex also heavily target binocular representations there, while avoiding the monocular representation. These results demonstrate that frontal neurons with visuospatial RFs provide a rich and specific pattern of feedback projections to V1 and multiple HVAs in mice. Despite this complexity, there was a prevalence of retinotopically-matched frontal projections to binocular V1 that could enable top-down control over this region of visual space.

Disclosures: A.D. Lien: None. **B. Haider:** None.

Poster

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Topic: E.06. Vision

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2R01EY023756

Title: Tuned recurrent inhibition controls feature-specific cortical accuracy

Authors: *M. B. OGANDO[1], L. ABDELADIM[2], K. SIT[3], H. SHIN[4], H. ADESNIK[5];
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Abstract: To infer structure from complex sensory scenes, animals must flexibly amplify relevant features while suppressing redundant or ambiguous information. Neocortical microcircuits may implement these computations through feature-specific recurrent connectivity, but whether these connections amplify or suppress tuned input and how they ultimately shape sensory processing remains hotly debated. In the visual cortex, identical visual stimuli can be either suppressed or facilitated by surrounding features depending on subtle differences in stimulus parameters. Likewise, recent experiments using targeted stimulation of orientation-tuned pyramidal cells (PCs) report either local amplification or suppression of co-tuned responses. How can the cortex produce such opposing forms of feature-specific computations? To address this central question, we used all-optical physiology to recreate sensory-evoked activity patterns in excitatory and inhibitory populations in the mouse visual cortex. In the absence of external visual input, photo-activation of orientation-tuned pyramidal cell ensembles generated visual-like activity in surrounding neurons, either evoking or suppressing their preferred stimulus. We tracked the feature-specific suppression to precise reciprocal like-to-like

connectivity between PCs and somatostatin interneurons (SSTs). Remarkably, augmenting SST activity during visual processing improved stimulus discriminability by suppressing ambiguous activity, but only when the visual input matched their preferred orientation. These results causally demonstrate that reciprocal, feature-specific SST-PC recurrent interactions can control the representational precision of external sensory inputs. More broadly, feature-aligned excitatory-inhibitory motifs may allow different strategies of contextual modulation to optimize inference by either cancelling or completing predictable activity, depending on the structure of the input and the network state.

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Poster

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Title: Feature-tuned synaptic inputs to somatostatin interneurons drive context-dependent processing

Authors: *W. HENDRICKS[1], M. SADAHIRO[1], D. MOSSING[1], J. VEIT[2], H. ADESNIK[1]; [1]Univ. of California, Berkeley, Berkeley, CA; [2]Physiol. I, Univ. of Freiburg, Freiburg, Germany

Abstract: Mapping neural computation onto the functional microarchitecture of sensory circuits is essential for understanding how brain circuits transform input signals into coherent percepts. In the visual cortex, one of the first computations required is segregation of figures from the background. A neural correlate of figure segmentation appears as early as primary visual cortex (V1): the response of V1 neurons is much greater to a stimulus within their receptive fields when it constitutes a “figure” than when that same stimulus forms part of the background, a phenomenon known as “figure/ground modulation”. Here, we combine single cell and population-level physiological recordings and perturbation methods to map this context-dependent cortical computation onto the synaptic microarchitecture of mouse V1. We demonstrate a precise, recurrent circuit between cortical pyramidal cells (PCs) and somatostatin (SST) inhibitory interneurons that “switches on” context-driven figure/ground modulation in V1. This pattern of “like-to-like” connectivity, previously only reported between pyramidal cells, explains SSTs’ visual encoding properties and their resulting impact on contextual modulation in V1. Though vasointestinal-peptide expressing (VIP) interneurons inhibit SSTs and could

therefore “switch-off” contextual modulation, we find that VIPs only play a supporting or modulatory role. These findings reveal key synaptic and circuit mechanisms that may underlie the earliest stages of scene segmentation in the visual cortex. Moreover, they raise the notion that feature-specific excitatory-inhibitory microcircuitry from PC to SSTs could be a general strategy that the cortex exploits to give rise to higher level computations.

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Poster

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Topic: E.06. Vision

Support: JCC postdoctoral fellowship
HHMI
NIH Grant 1R01NS123912

Title: The molecular identity of visual cortical neurons receiving thalamic input

Authors: *X. GE[1], X. WEI[2], B. RUAN[1], Q. WU[1], N. TSAI[1], X. DUAN[3], M. SCANZIANI[3]; [1]UCSF, San Francisco, CA; [2]Physiol., UC San Francisco, San Francisco, CA; [3]Univ. of California, San Francisco, San Francisco, CA

Abstract: Thalamic afferents are the main conduit of sensory information to the cortex, where they selectively contact specific cortical neurons to set the stage for cortical sensory processing. Despite recent advances in the transcriptomic characterization of cortical cells, the molecular identity of cortical neurons targeted by thalamic afferents is still poorly understood. Using the mouse visual cortex as a model, we combined anterograde transsynaptic tracing with spatial transcriptomics to identify the molecular signatures of cortical neurons receiving input from each of the two main thalamic afferents nuclei to visual cortex, the dorsolateral geniculate nucleus and the pulvinar. Our data reveal the transcriptional profiles associated with each input pathway and provide new insight into the cellular organization of bottom-up and top-down information flow in visual cortex.

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Poster

PSTR072: Visual Cortex: Circuits

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Program #/Poster #: PSTR072.11/J7

Topic: E.06. Vision

Title: Spatiotemporal stimulus information is systematically encoded in V1 and is regulated by Parvalbumin and Somatostatin interneurons

Authors: *S. POST[1], W. MOL[2], N. RAHMATULLAH[3], A. GOEL[4];
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Abstract: Spatial features of visual encoding have been well studied, but little research has investigated the temporal features of visual encoding. To address this, we recorded Pyramidal, Somatostatin (SST) and Parvalbumin (PV) activity in L2/3 of V1 using 2p Ca^{2+} imaging as mice were passively exposed to sequences of time varying stimuli. Sequences were composed of 3 interleaved 200 ms gratings, in which the final 2 gratings and the duration between them were identical across all sequences. The orientation of the first grating and the period between the first and second grating were systematically varied. We found that encoding of the final two gratings in the sequences, despite being identical, systematically varied as a function of both the duration between the first and second grating as well as the orientation of the leading stimulus. In effect, there was a transient memory trace in V1 which depended upon the preceding spatiotemporal information. This differential encoding results from different state space trajectories and is regulated by both PV and SST neurons, likely due to each's short term plasticity profiles. Our results provide a mechanism for how sequences of stimuli are encoded in a primary sensory area, such as in music or language, which may differ from the encoding schema of discrete stimuli.

Disclosures: S. Post: None. **W. Mol:** None. **N. Rahmatullah:** None. **A. Goel:** None.

Poster

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Program #/Poster #: PSTR072.12/J8

Topic: E.06. Vision

Title: Circuit Mechanisms of Dynamic Sensory Encoding and Sensory Plasticity Across Learning in Primary Visual Cortex

Authors: *W. MOL, IV[1], S. POST[2], N. RAHMATULLAH[3], A. GOEL[4];
[1]Univ. of California Riverside, Riverside, CA; [2]UC Riverside, Riverside, CA; [3]Univ. of California, Riverside, Riverside, CA; [4]Psychology, UCR, Riverside, CA

Abstract: In our environment, we are constantly presented with sensory information that predicts coincident or future outcomes and, as such, has a bearing on our behaviors. Adapting to novel stimuli, constructing new associative relationships, and contextually processing familiar

stimuli require feature-specific selectivity and the construction of an internally generated model. Growing evidence has shown primary visual cortex (V1) to be adaptable and plastic, changing the nature of its population response and its representation of stimuli with both repeated exposure and across learning. Models like predictive processing posit a statistical updating of sensory responses, both as a consequence of past experience and of stimulus contingency. A key underlying principle of predictive processing, efficient coding, and the phenomena of V1 dynamically shifting pair well, positioning the earliest point in the cortical visual stream as a primary substrate for learned sensory contingencies. V1, in addition to bottom-up sensory information, receives rich input from top-down modulatory areas like the anterior cingulate cortex (ACC). This ACC -> V1 input has been shown to carry information about stimulus-specific valence and putative prediction errors, as well as being shown necessary for rule updating and attenuation to distractors in specific tasks. In this work, we use 2-photon calcium imaging of layer 2/3 of V1, targeting pyramidal cells and somatostatin-expressing interneurons (SST), and axon terminals of ACC in superficial layers of V1 throughout task learning. Our novel head-fixed task is a go/no-go sensory discrimination paradigm consisting of stimuli constructed by sequences of four different angles of sinusoidal drifting orientations. The first and last of these elements are relevant for sensory discrimination, and are reversed between rewarded and unrewarded sequences, and the middle two are of a constant identity and carry no information about stimulus valence. Examining responses to these sequences—and their individual elements—reveals cell-type specific shifts, contributing to overall gradual changes in population dynamics as learning progresses, as well as changes in interleaved task-disengaged passive exposure sessions.

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Poster

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Topic: E.06. Vision

Support: DFG 524025372

Title: Heterogeneity of inhibitory synapse placement on L2/3 pyramidal neurons in mouse visual cortex

Authors: *B. SCHMERL[1], J. R. BOIVIN[2], Y. SHARAV[4], M. LONDON[6], I. SEGEV[5], E. NEDIVI[3,7];
[1]The Picower Inst. for Learning and Memory, [2]The Picower Inst. for Learning & Memory, [3]MIT, Cambridge, MA; [4]Hebrew Univ., Jerusalem, Israel; [5]Hebrew Univ., Jerusalem, ; [6]The Hebrew Univ. of Jerusalem, Jerusalem, ; [7]Picower Inst. for Learning and Memory, Massachusetts Institute of Technology, MA

Abstract: It is increasingly evident that inhibitory circuits play a key role in neurological deficits as well as experience-dependent plasticity. Yet, we know little regarding how inhibitory synaptic subtypes may differentially contribute to this role. Inhibitory signaling in the cortex originates from GABAergic interneurons, which are broadly divided into three classes, based on their distinct expression of Somatostatin (SST), Parvalbumin (PV) and certain Serotonin-receptors (5HT3aR). While there is a body of research showing that synaptic transmission differs functionally between these classes, studies examining their cellular targeting onto pyramidal neurons are often limited in resolution and/or coverage of the dendritic arbor.
Here we used Cre-dependent Synaptophysin-TdTomato expression to comprehensively label inhibitory subtype-specific presynaptic boutons synapsing onto sparsely labelled, YFP-cell filled L2/3 pyramidal neurons in binocular mouse visual cortex. Synaptic contacts were then validated and proteomically characterized with epitope-preserving analysis of proteome (eMAP), a combination of tissue clearing and expansion microscopy.
We were thus able to map the sub-cellular distribution of inhibitory synapses across the whole dendritic arbor of pyramidal neurons in relation to their inhibitory afferent identity. We found that contrary to popular belief, SST synapses account on average for only 60% of dendritic inhibitory synapses, and that PV inhibitory synapses in addition to innervating the soma also account for another 30% of dendritic inhibitory synapses. Further, we see that subtype-specific synapses are segregated and heterogeneously distributed across individual dendrites and between cells. This work is an important step in defining subtype-specific distribution of inhibitory synapses, towards understanding their distinct roles in dendritic computation and circuit function.

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Poster

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Topic: E.06. Vision

Support: DFG Emmy Noether VE 938/2-1

Title: Context-dependent interareal synchronization across mouse visual cortex

Authors: *C. RAMANATHAN[1,2], D. ERIKSSON[1], J. VEIT[1,2];
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Abstract: Most brain functions rely on constant interactions among specialized brain areas. In primates, synchronized oscillations in distinct frequency bands have been suggested to facilitate the routing of feedforward and feedback signals across hierarchically organized visual areas. However, the underlying mechanisms remain largely unknown. Previous studies in mice

identified a context-dependent low gamma rhythm (~20-35 Hz) depending on the activity of local somatostatin (SST) interneurons in V1. Our analysis of the open-source Allen Institute Neuropixels dataset revealed the presence of this low-gamma oscillation throughout the entire visual cortical hierarchy at varying strengths across cortical layers. To understand the properties and synchronisation of this oscillation across visual cortical areas, we performed simultaneous multi-areal Neuropixels recordings combined with causal optogenetic perturbations in retinotopically-matched locations in V1 and LM of awake, head-fixed mice. The low frequency gamma rhythm exhibits distinct stimulus dependencies and a precise laminar synchronization profile in both V1 and LM. Interareal coherence and Granger causality indicate bidirectional communication revealing multiple sub-modules of synchronized channels distributed across the superficial and deep layers of V1 and LM. Pairwise phase consistency analysis shows prominent spike-field coupling of LM neurons to the V1 rhythm and of V1 neurons to the LM rhythm across all layers. Optogenetic silencing of V1 led to a decline in low gamma activity in LM. Furthermore, the inhibition of SST interneurons in LM also reduced low gamma activity within LM, implying a combined contribution of feedforward entrainment and a local PING mechanism in the generation of this rhythm in LM. Looking further into the role of feedforward drive, reducing gamma power in V1 by suppressing local SST interneurons also led to a prominent decrease in gamma power in LM, despite firing rates being markedly increased in both V1 and LM, showing that just the increased feedforward drive without the specific synchronization pattern is not sufficient to synchronize the downstream LM population.

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Poster

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Support: Max Planck Society

Title: Population-scale representations of object length in awake tree shrew V1

Authors: *J. BARCHINI, D. FITZPATRICK;
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Abstract: Stimulus length representation and discrimination are vital visual skills that many animals rely on to successfully navigate their environment and make important decisions. A psychophysical signature of this ability is the Just Noticeable Difference (JND): the smallest distinguishable difference in length between two stimuli. The JND in humans and non-human primates reveals a sensitivity to small differences between small stimuli, but larger differences between larger stimuli. Tree shrews are highly visual mammals, with a visual system that shares features of organization and function with that of the primate. In this study we show that tree

shrews are capable of fine discrimination of bar lengths, and preliminary data suggests that their JND increases in proportion to stimulus length, akin to what has been escribed in the literature. Furthermore, using 2-photon calcium imaging in tree shrew primary visual cortex (V1) we looked for neural correlates of stimulus length representation. We observed robust and prevalent surround suppression at the single-cell level in awake head-fixed animals. However, unlike features that are seeded in the structure of a neuron's classical receptive field (e.g. orientation and spatial frequency tuning), we found that length tuning proper is poorly resolved from the responses of individual cells. Additionally, using dimensionality reduction and artificial neural networks, we showed that length representation is also not a locally expressed feature, but can rather be derived from activity at the scale of the extended network. These observations establish a new role for a long-studied feature in single cells (i.e. surround suppression) in priming the larger network to represent object length.

Disclosures: J. Barchini: None. **D. Fitzpatrick:** None.

Poster

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Title: Laminar organization of temporal codes for stimulus continuity and their development in ferret V1

Authors: *A. A. LEMPEL, D. FITZPATRICK;
Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: A fundamental contribution of the visual cortex is the integration of stimulus information with contextual cues to support experience-based inference. While the circuit mechanisms underlying contextual integration in mature animals remain poorly understood, even less is known about how these functions develop. To study this, we focused on the primary visual cortex (V1) of ferrets, a model well-suited for studying vision development in modular cortical circuits. As a first step, we asked how different cortical layers encode the continuity of a stimulus with its surrounding context in visually experienced ferrets (postnatal day (P)40-55). We recorded spiking responses across layers to a drifting grating centered on the receptive field that was either continuous with a surrounding grating, forming a full-field stimulus, or displaced 180 degrees in spatial phase relative to the surround (discontinuous). Preliminary data indicate that response strength and orientation tuning were similar across layers for the two stimulus conditions, but neurons in layers (L)2/3 and L5/6 exhibited robust spike-local field potential (LFP) coherence that shifted in frequency composition between continuous and discontinuous

stimuli. These findings suggest that neurons in L2/3 and L5/6 exhibit a temporal code of stimulus continuity that is absent in L4. Notably, L2/3 and L5/6 spike-LFP coherence for continuous gratings was weaker and exhibited different frequency spectra in visually naïve ferrets (P27-32), indicating that this coding develops in the first two weeks of vision. A possible mechanism underlying the representation of continuity is that L2/3 and L5/6 contrast center-stimulus-driven inputs from L4 with feedback and lateral inputs representing the surround stimulus. To test this, we performed partial optogenetic suppression of L4 activity during stimulation with continuous gratings. In experienced animals, suppressing L4 activity reduced visual responses in L2/3, while L5/6 remained strongly responsive and orientation-tuned. However, spike-LFP coherence in L2/3 and L5/6 shifted to more closely resemble that elicited by discontinuous stimuli. These results indicate that L4-derived inputs are essential for the temporal coding of stimulus continuity, but not for basic tuning properties in deeper layers. Our data provide novel insights into how contextual information is encoded in spatiotemporal activity patterns in the visual cortex of an animal well suited for studying experience-driven visual development. Future experiments will further investigate this temporal code of stimulus continuity and its emergence following the onset of visual experience.

Disclosures: A.A. Lempel: None. **D. Fitzpatrick:** None.

Poster

PSTR072: Visual Cortex: Circuits

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR072.17/J13

Topic: E.06. Vision

Title: Sequence of excitatory and inhibitory network formation during initial development of cortical circuits

Authors: *A. GRIBIZIS[1], D. FITZPATRICK[2];
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Abstract: A characteristic feature of early cortical circuit development is spontaneous patterns of co-activity that contribute to the early organization of synaptic networks subsequently refined by sensory experience. The mature visual cortex in highly visual animals is characterized by iterated clusters of similarly responding neurons, or modules, that provides an excellent model system for exploring the mechanisms underlying the development of functional network organization. The sequence of early events and the cell types that lead to the initial emergence of modular network structure remain unknown. Here we employ multi-color imaging to chronically measure calcium signals in genetically targeted cell types in tree shrew visual cortex starting a week before eye opening. We explore the interaction between excitatory and inhibitory cellular networks during the initial development of functional modular networks in cortex. We find that patterned spontaneous waves are broad and unspecific early in the developing visual cortex in both cell types. Then, excitatory populations rapidly transform within a day into a modular

coactivation of multiple patches extending millimeters across the cortical surface. Inhibitory networks follow excitatory patterns within a few days.Interestingly during this period of early visual system development, linear wavefronts of spontaneous activity also sweep across the retina and downstream visual pathways before eye opening in many species. Early on, we find a correlation between wavefront trajectory and modular pattern identity in excitatory cells, with orthogonal wavefronts activating complimentary modular patterns. These wavefronts create an axial bias in spontaneous modular pattern associations, resembling mature horizontal connectivity before sensory experience. Inhibitory cells again trail excitatory populations, initially coactive with only nearby regions and only developing long-range correlations shortly before eye-opening. The results presented here suggest a sequence of cell-type recruitment into initial cortical networks, where excitatory cell types lead inhibitory cells in their network involvement and identity.

Disclosures: A. Gribizis: None. **D. Fitzpatrick:** None.

Poster

PSTR072: Visual Cortex: Circuits

Location: SDCC Halls B-H

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Program #/Poster #: PSTR072.18/J14

Topic: E.06. Vision

Support: NIH Grant EY034503

Title: Intracellular analysis of receptive fields in rabbit visual cortex during naturally occurring alert and non-alert states

Authors: *N. RAFFONE, M. CHISTYAKOVA, Y. BERESHPOLOVA, M. A. VOLGUSHEV; Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT

Abstract: The visual system effectively extracts vital information from the environment under rapidly changing conditions, both external and internal. External are changes of the visual world around us, and internal are changes of the brain state. Throughout the day we have hundreds of shifts in arousal that can alter operation of the visual system. Understanding such changes is vital for understanding how visual perception is affected by the brain state. Previous studies of cortical receptive fields (RF) have shown diverse effects of brain states on their size and spatiotemporal dynamics. However, such studies are limited by either the use of varying levels of anesthesia in anesthetized preparations, or by using tasks requiring the state of heightened arousal, e.g. during maintaining precise eye fixation or locomotion. High-attention state represents an extreme on a continuum of states of alertness, while everyday perception mostly takes place during lower levels of alertness and attention. Here, we employed intracellular recordings to study the spatiotemporal structure of RFs of V1 neurons during naturally occurring alert and drowsy states to address this question. We took advantage of the head-restrained rabbit that can sit quietly for hours and exhibit very little eye movements while spontaneously and

naturally transitioning between alert, non-alert/drowsy, and sleep states. Brain states were defined by the characteristic signatures of EEG recorded in the somatosensory cortex and hippocampus. Eye position was monitored using pupillometry and/or recording of activity from superior colliculi neurons with small RFs. Using subthreshold membrane potential dynamics and spiking, we measured responses of cells to sparse-noise stimulation over multiple transitions between alert and non-alert/drowsy brain states. Spatiotemporal RF profiles were calculated using reverse correlation method. Our analysis showed that in regular-spiking (RS) cells, RF subfields were smaller during alert than during non-alert states. During non-alert state, ON and OFF subfields also showed higher degree of overlap. Analysis of the temporal RF profiles revealed that the duration of ON and OFF responses was shorter during alert than non-alert state. In fast-spiking (FS) neurons, the strength of ON and OFF responses was more balanced than in RS cells, and RFs were less affected by state changes. Our findings show that alertness affects spatiotemporal properties of RFs in rabbit V1, and the effects are different in RS and FS neurons.

Disclosures: N. Raffone: None. **M. Chistyakova:** None. **Y. Bereshpolova:** None. **M.A. Volgushev:** None.

Poster

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Program #/Poster #: PSTR072.19/J15

Topic: E.06. Vision

Support: R01EY034503
R01EY028905
R21EY030291

Title: Single thalamocortical axons form facilitating synapses onto visual corticotectal neurons and depressing synapses onto neighboring fast-spike interneurons

Authors: *C. SU[1], H. A. SWADLOW[2], Y. I. BERESHPOLOVA[2];
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Abstract: Thalamocortical synapses play a critical role in driving cortical sensory responses, and differentially governing cortical excitability through their dynamic synaptic properties. It is well established that the thalamus provides potent feed-forward monosynaptic input to fast-spiking PV+ GABAergic interneurons (suspected inhibitory interneurons, SINs) of layers 4 and 6 through synapses that exhibit short-term depression. Short term synaptic depression has also been documented in studies of thalamocortical synapses onto cortical excitatory neurons of layer 4, but much less is known about synaptic dynamics of thalamocortical inputs to different classes of layer 5 neurons. We recently showed that the dorsal lateral geniculate nucleus (LGN) provides potent monosynaptic drive to layer 5 pyramidal corticotectal (CTect) neurons in the primary visual cortex, and we have also shown that these CTect neurons form strongly depressing

synapses onto their targets within the superficial layers of the superior colliculus. Yet whether the thalamocortical synapse onto these CTect pyramidal neurons exhibit short-term depression or facilitation remains unknown. Here, we investigate, in awake rabbits, short-term synaptic dynamics of synapses formed by single LGN axons onto two classes of layer-5 cells: excitatory CTect neurons and neighboring putative fast-spike inhibitory interneurons (SINs). We found that single LGN axons exhibit distinct cell-type specific release dynamics in layer 5. Whereas LGN synapses onto SINs all exhibited synaptic depression, LGN synapses onto neighboring CTect neurons showed synaptic facilitation. Thus, whereas the responsiveness of CTect neurons sharply increased with sustained presynaptic LGN firing, the responsiveness of the inhibitory SINs fell dramatically, suggesting an enhanced role for CTect output under conditions of maintained LGN discharge. Notably, we have previously shown that maintained (sustained) firing of LGN neurons to maintained visual stimulation is highly dependent on the state of arousal, suggesting that rapid fluctuations in brain state are accompanied by corresponding fluctuations in the role of the CTect impact on visual processing.

Disclosures: C. Su: None. **H.A. Swadlow:** None. **Y.I. Bereshpolova:** None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR073.01/J16

Topic: E.06. Vision

Support: 5R00EY031333-04

Title: Reconstruction of visual images from mouse retinal ganglion cell spiking activity using convolutional neural networks

Authors: *S. CHEN[1], D. BABINO[2];
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Abstract: Reconstruction of Visual Images from Mouse Retinal Ganglion Cell Spiking Activity Using Convolutional Neural Networks
Sichen Chen
Upstate Medical University
Advisor/PI: Darwin Babino, PhD
Abstract:
Purpose: To assess the ability of trained convolutional neural networks (CNN) to reconstruct novel facial images from retinal ganglion cell (RGC) spiking data to better elucidate how visual information is processed and conveyed by the retina, and to develop a model for reconstructing images from neural activity.
Methods: Ex-vivo preparations of mouse retina were mounted on a high-density multielectrode array (MEA) to record and process RGC spiking activity in response to facial image stimuli from

a digital projector. Spiking data from RGCs in response to 2,240 of 2,800 images were used to train a U-Net-style CNN (ResUNet) to reconstruct the images, with the remainder used for validation and testing.
Results: The CNN was able to reconstruct novel facial images from RGC signaling, and the ResUNet model outperformed multilayer perceptron (MLP) and linear models. Contributions to image reconstruction varied by RGC subtypes, with high-firing rate cells contributing the most. ON and OFF cell subtypes provided partially redundant information, but reconstruction of specific structures may rely more heavily on input from one or the other cell type. Preferential utilization of local RGC signaling in reconstruction of specific features was observed, although additional contributing cells may be in remote locations.
Conclusions: Artificial neural networks are capable of learning multicellular sensory neural encoding, and we present a viable model for understanding visual information encoding.
Translational relevance: This study demonstrates the potential of machine learning in bridging biological and artificial neural networks, which may influence the development of vision restoration technologies.

Disclosures: S. Chen: None. **D. Babino:** None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

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Program #/Poster #: PSTR073.02/K1

Topic: E.06. Vision

Support: NIH R01 EY018849
NIH T32 EY007031
NIH F31 EY037124

Title: Phase-invariant integration of luminance and color by V1 complex cells

Authors: *L. BUN[1], G. HATANAKA[2], T. KIM[2], C. DYLLA[2], W. BAIR[2], G. D. HORWITZ[2]; [1]Bioengineering, [2]Neurobio. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Most neurons in the primary visual cortex (area V1) can be divided into two groups: simple and complex. Unlike simple cells, complex cells are insensitive to the phase of a grating stimulus. Additionally, many complex cells in primates respond to both luminance and chromatic contrast. But how complex cells integrate luminance and chromatic signals is poorly understood. One possibility is that complex cells are sensitive to the relative phase between superimposed luminance and chromatic gratings despite being insensitive to the phase of either one alone. If so, then complex cells may play a role in humans' greater sensitivity for spatially aligned gratings. To test this idea, we presented monkeys with superimposed luminance and chromatic gratings that varied in contrast and were aligned (0 and 180°) or offset (90 and 270°) (Fig. 1a). We recorded from neurons in macaque V1 with Neuropixels probes, identified complex cells offline,

and, for each one, determined the contrast of each stimulus that would drive a common firing rate. If complex cells underlie psychophysical results, then we expect them to have lower isoresponse contrasts when gratings are aligned. Alternatively, complex cell responses may follow the predictions of the color energy model. The model squares and sums the outputs of pairs of filters that are 90° out-of-phase with different color tunings (Fig. 1b). Under this model, the isoresponse contrasts obey a mathematical relationship (Fig. 1c) such that the isoresponse contrasts of the offset stimuli fall between those of the two aligned stimuli. We found that, for most complex cells, the relationship between isoresponse contrasts conformed to the energy model prediction. Across neurons, no significant bias away from this prediction was detected. Thus, unlike human observers, V1 complex cells are not preferentially sensitive to aligned luminance and chromatic modulations. This suggests that either sensitivity to relative phase is mediated by individual neurons in higher cortical areas (e.g. V2 or V4) or that it is encoded by a population of V1 neurons.

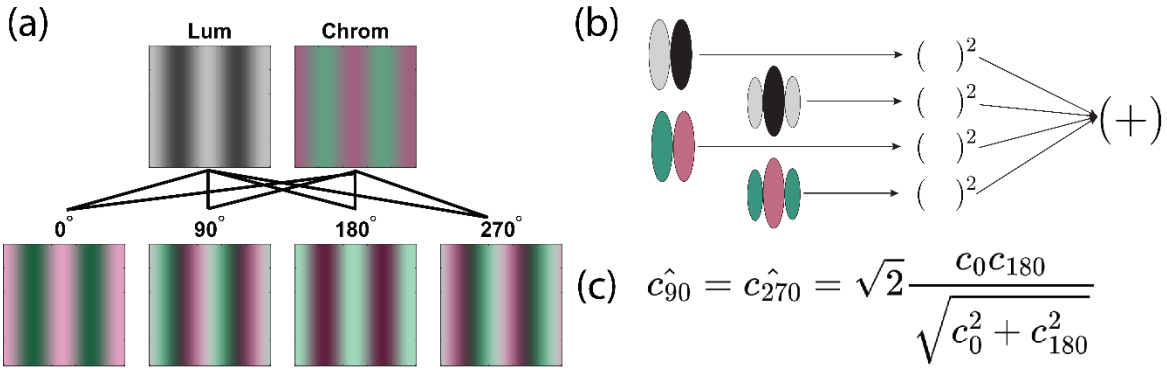


Figure 1. (a) Stimuli are superimposed luminance and chromatic gratings with varying relative phase. (b) The color energy model is composed of pairs of filters that are 90° out-of-phase with different preferred colors whose outputs are squared and summed. (c) The color energy model predicts that the isoresponse contrasts of the 90 and 270° stimuli are equal to the product of isoresponse contrasts of the 0 and 180° stimuli divided by their root mean square.

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Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Program #/Poster #: PSTR073.03/K2

Topic: E.06. Vision

Support: IA/S/18/2/504003

Title: Saturated red color elicits stronger gamma oscillations in the primate visual cortex

Authors: *A. BISWAS;
Indian Inst. of Sci., Bangalore, India

Abstract: Gamma oscillations (30-150 Hz) are prominent neural rhythms critical for cognitive functions and are often disrupted in neurological disorders. In the visual cortex, gamma activity is tightly linked to stimulus features such as contrast and spatial frequency, making it a useful marker of neural circuit function. Previous studies in non-human primates have shown that red stimuli evoke stronger gamma responses in the primary visual cortex (V1) than even optimal achromatic gratings. However, Stauch et al. (2022), using magnetoencephalography (MEG) in humans, reported no difference in gamma power between red and green stimuli when L-M cone contrast was equalized—revealing a potential discrepancy between invasive and non-invasive findings. To address this, we simultaneously recorded local field potentials (LFPs) from V1 (n=3 macaques) and V4 (n = 1 macaque), along with scalp electroencephalography (EEG), while the monkeys-maintained fixation on a central point. We presented color stimuli from three color spaces: HSV (maximally saturated monitor-visible colors), DKL (isoluminant colors), and CIE (high saturation with equal luminance across hues). In HSV space, all three monkeys showed a clear red preference in V1 gamma activity, replicating prior findings. This preference was reduced in V4 and EEG recordings. In DKL space, two of the three monkeys also showed a red preference for hues with higher L-M cone contrast, while one monkey did not show clear preference. EEG responses also had reduced red preference. In CIE space—where colors were saturated and matched in luminance to the background—V1 gamma oscillations remained strongest for red compared to other hues. These results show that red-selective gamma responses in V1 are dependent on stimulus saturation and are attenuated in downstream areas and non-invasive signals. The reduced hue specificity in EEG/MEG likely reflects spatial summation across cortical regions, helping reconcile prior discrepancies. Our findings highlight the role of color saturation in modulating gamma oscillations and emphasize the importance of stimulus design and recording modality in the study of neural rhythms.

Disclosures: A. Biswas: None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Topic: E.06. Vision

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ARC grant DP210103865
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NHMRC grant APP1185442

Title: Contrast sensitivity in the marmoset monkey

Authors: *G. MA[1], L. INGER[2], S. L. CLOHERTY[3], M. G. ROSA[2], N. ATAPOUR[2], M. A. HAGAN[2];
[1]Dept. of Physiol., [2]Dept. of Physiol. and Biomedicine Discovery Inst., Monash Univ., Clayton, Australia; [3]RMIT Univ., Melbourne, Australia

Abstract: Sensitivity to luminance contrast is one of the basic measures of visual capacity and is known to vary according to both spatial and temporal frequency of a stimulus. Macaque monkeys exhibit a contrast sensitivity function comparable to humans with respect to spatial and temporal frequency. However, the contrast sensitivity of marmoset monkeys (*Callithrix jacchus*), an increasingly important model for visual neuroscience, has not been thoroughly characterised. Here, we measured contrast sensitivity in three marmosets using a saccade-based, 4-alternatives forced choice (4-AFC) task. Stimuli consisted of drifting sine-wave gratings with varying spatial (0.1-8 cpd) and temporal frequencies (0.5-32 Hz), windowed with a gaussian envelope (σ = 0.5°), presented at 5° eccentricity on a uniform grey background matched to the mean luminance of the gratings (11.26 cd/m^2). Marmosets were rewarded for making a saccade to within 2.5° of the grating. Detection thresholds were determined by fitting a Weibull distribution to the performance data for each monkey. Our results demonstrate that marmosets can reliably detect grating stimuli across the tested frequency ranges, with the peak contrast sensitivity ranging from 26.87 to 55.41 at spatial frequencies of 0.47 - 0.68 cpd and temporal frequencies of 3.13 - 5.19 Hz. Similar to humans and macaques, the contrast sensitivity profiles for both spatial and temporal frequency follow log-parabolic trends (r^2>0.7, p<0.05, F-test), although marmosets exhibit lower peak contrast sensitivity values and narrower detectable ranges. Interspecies differences in contrast sensitivity may be attributed to species-specific anatomical and functional specialisations in retinal cellular pathways and cortical processing mechanisms. Characterising the contrast sensitivity function provides an essential baseline for future vision-based psychophysical and behavioural studies in marmosets.

Disclosures: G. Ma: None. **L. Inger:** None. **S.L. Cloherty:** 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.; ARC grant DP210103865, NHMRC grant APP1185442. **M.G. Rosa:** 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.; NHMRC grant 2041019, ARC grant DP210103865. **N. Atapour:** 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.; NHMRC grant 2019011. **M.A. Hagan:** 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.; ARC grant DP250104165, NHMRC grant APP1185442.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

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Topic: E.06. Vision

Support: NIH Grant R00EY031795
McDonnell Center for Systems Neuroscience /Small Grants Program
NSERC Canada Grant RGPIN-2019-03938

Title: Lightness is computed downstream from simultaneous contrast in area V4

Authors: *F. DIDEHVAR[1], P. CAVANAGH[3], T. P. FRANKEN[2];
[1]Washington Univ. Sch. of Med., SAINT LOUIS, MO; [2]Neurosci., Washington Univ. Sch. of Med., St. Louis, MO; [3]Psychological and Brain Sci., Glendon Col., Toronto, ON, Canada

Abstract: Humans readily perceive reflectance as lightness, that is, how white, gray, or black an object is. This is remarkable because the amount of light received by our eyes from an object (luminance) can vary by a factor of up to 10^8 due to illumination, whereas reflectance varies only by a factor of up to about 30. Even though illumination is typically non-uniform in natural scenes, vision maintains a stable perception of lightness: lightness constancy. How this works remains poorly understood. Low-level theories suggest that this results from simple spatial filtering early in the visual pathway. In contrast, mid- and high-level theories propose that higher stages analyze scene illumination or luminance to compute lightness. To distinguish between these theories, we used Neuropixels to record from columnar clusters of neurons in Area V4 of two macaques while the animals were fixating on scenes. We presented Shadow scenes (checkerboards partially covered by shadow), Paint scenes (derived from Shadow scenes, but destroying the shadow by averaging luminance within each square), and Shuffled scenes (created by randomly permuting squares in the Paint scenes). We centered the classical receptive fields in one square in the shadow (or in the corresponding square in Paint and Shuffled scenes) and varied the luminance difference between the squares of the board. We trained random forest decoders to decode luminance from neural responses to Paint scenes, and tested these decoders on unseen data from either Shadow (*test-shadow*) or Paint scenes (*test-paint*). We defined the lightness signal as the difference in predicted luminance between test-shadow and test-paint. Using the same method on Paint vs. Shuffled scenes generated a simultaneous contrast signal. Our data show significantly positive lightness and simultaneous contrast effects in V4 (n = 10 penetrations), consistent with perception. By shuffling the data for one neuron at a time between training and testing the decoders, we can identify the neurons that contribute most to these signals. These neurons responded to Shadow and Paint scenes with identical luminance as if the Shadow scene had higher luminance. Notably, the lightness signal was significant only in extragranular (non-input) cortical layers, whereas the simultaneous contrast signal was also significant in the granular (input) layer, suggesting that simultaneous contrast is available in the input to V4 while lightness arises in V4. These results indicate that V4 neurons encode lightness rather than luminance. The laminar distribution suggests that lightness is computed downstream from simultaneous contrast, supporting mid- and high-level theories

Disclosures: F. Didehvar: None. **P. Cavanagh:** None. **T.P. Franken:** None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Program #/Poster #: PSTR073.06/K5

Topic: E.06. Vision

Support: National Science and Technology Innovation 2030-Major Project 2022ZD0204803
Natural Science Foundation of China Grant NSFC32271081

Title: Neural mechanisms for canonical object hues in color-selective regions of macaque IT cortex

Authors: *K. WANG[1], X. LIU[3], L. YIPENG[1], Y. BI[4], P. BAO[2];
[1]Peking Univ., Beijing, China; [2]Peking Univ., Peking, China; [3]Peking-Tsinghua Ctr. for Life Sciences, Peking Univ., Beijing, China, Beijing, China; [4]State Key Lab. of Cognitive Neurosci. and Learni, Beijing, China

Abstract: Recent fMRI studies have revealed that macaque color patches, particularly those near area TEO, encode object color knowledge—for example, associating grayscale strawberries with the color red—through perceptually grounded neural activity (Zhao et al., 2024). However, how such knowledge is instantiated at the level of individual neurons and local microcircuits remains unknown. Moreover, because fMRI lacks temporal resolution and directional specificity, it remains unclear whether these representations arise from feedforward sensory encoding or top-down feedback from higher-order areas.To address these questions, we recorded extracellular activity using Neuropixels probes from the middle color-selective region of the macaque inferior temporal (IT) cortex while animals passively viewed grayscale objects, chromatic gratings, and real- versus false-colored versions of familiar objects.At the population level, color-selective sites supported cross-stimulus decoding: responses to color gratings predicted the canonical color of grayscale objects. Representational similarity analysis further revealed that the population response pattern to a given color grating more closely resembled that elicited by grayscale objects with matching canonical colors than those with mismatched hues. At the single-unit level, many neurons exhibited conjunctive tuning for hue and object shape, suggesting that individual cells encode integrated object-color representations.Importantly, population responses could reliably distinguish real versus false-colored object images (e.g. red watermelon vs. green strawberry), and this effect was greatly diminished for abstract geometric shapes (e.g., red triangle vs. green circle), reinforcing that the observed selectivity reflects genuine object-color knowledge rather than simple color-shape associations.Temporal analyses revealed that these effects peaked during the early sensory-driven response period, suggesting that object-color knowledge in this region likely arises from feedforward processing rather than top-down modulation.Together, Our results provide direct neural evidence for how the primate brain links perceptual color information with stored knowledge about objects, offering a mechanistic account of semantic memory grounded in sensory experience.

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Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Program #/Poster #: PSTR073.07/K6

Topic: E.06. Vision

Support: NEI grant EY035085
NEI grant EY035838

Title: Generative Phenomenology of form perception: Perceptograms and cortical models for amblyopic form distortions

Authors: A. MARUYA, B. RAMAKRISHNAN, F. OLIANEZHAD, J. WANG, J.-M. ALONSO, ***Q. ZAIDI**;
Grad. Ctr. for Vision Research, SUNY Optometry, New York, NY

Abstract: We introduce Generative Phenomenology - making viewable images of people's perceptions (Perceptograms) and generating the images from cortical models - as a powerful technique for understanding the neural bases of perception. Amblyopia, a disorder of spatial vision, provides a perfect case because signals from the two eyes go through partly different cortical neurons, and a majority of amblyopes report form distortions when viewing sinusoidal gratings through their amblyopic eye (AE), but not through the fellow eye (FE). The drawings of these distortions fall into 7 characteristic classes. To acquire perceptograms with higher fidelity, we used a dichoptic display to show the amblyopic eye (AE) a 3 dva test grating (6, 9, or 12 cpd; 0, 45, 90 or 135° orientation; ON or OFF polarity), while the fellow eye (FE) was shown 8 surrounding patterns: the test grating and 7 plaids from the distortion classes. Observers selected the pattern that most resembled the perceived distortion. The match was then refined by adjusting contrast, frequency, phase, and orientation of the component gratings until the two percepts matched exactly. Plaid perceptograms provided exact matches to 92.6% of distortions.

Our linking hypothesis is that the cortical representation of the test grating through AE neurons matches the perceptogram through FE neurons. For each condition, we passed all 24 gratings paired with their perceptograms through steerable filters modeling normal V1 and tested if a linear transformation $\tilde{L}_i$ for each filter i can account for the cortical deficits. $\tilde{F}_{s,i}$ and $\tilde{F}_{d,i}$ are the Fourier transforms of AE filter responses to the gratings and FE filters to the perceptograms, and $\tilde{b}$ is independent noise:

$$\widetilde{r_{f,i}} = \widetilde{F_{d,i}} + \widetilde{b_f}, \quad \widetilde{r_{a,i}} = \widetilde{L_i}\,\widetilde{F_{s,i}} + \widetilde{b_a}$$

Setting $\widetilde{r_{f,i}} \approx \widetilde{r_{a,i}}$, we calculated $\mathrm{P_F}$ the power-spectrum of $\widetilde{F_{s,i}}$ and used $P_{r,F}$ the cross-spectrum to derive $\tilde{L}_i$ irrespective of the noise:

$$P_{r,F} = <\widetilde{r_{a,i}}, \widetilde{F_{s,i}}^{*}> = <\widetilde{r_{f,i}}, \widetilde{F_{s,i}}^{*}> = \widetilde{L_i}\,\mathrm{P_F} \Longrightarrow \widetilde{L_i} = P_{r,F}\mathrm{P_F}^{-1}$$

Passing gratings through the discrete set of derived AE filters generated the perceptograms accurately for all conditions. Some filters had transformed spatial frequency or orientation preference, but others reflected complex changes in RFs and output correlations. RFs showed increased spatial extent, consistent with the increase in contrast sensitivity with field size in amblyopia. The AE filters explained amblyopic deficits in perceiving sinusoidally modulated circular contours and were consistent with orientation tuning estimated from psychophysical reverse-correlation experiments. Changes in neuronal receptive fields thus have profound effects on perception, to the extent that observers can see more features than are present in the viewed stimulus.

Disclosures: A. Maruya: None. **B. Ramakrishnan:** None. **F. Olianezhad:** None. **J. Wang:** None. **J. Alonso:** None. **Q. Zaidi:** None.

Poster

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Program #/Poster #: PSTR073.08/K7

Topic: E.06. Vision

Support: NEI grants EY035085
NEI grants EY035838

Title: Comparing apples to oranges from photoreceptors to inferotemporal cortex via neural manifolds

Authors: *Y. XU[1], A. MARUYA[2], B. R. CONWAY[3], Q. ZAIDI[2];
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Abstract: The neural processing of color begins with light absorption in 3 classes of cone photoreceptors (L, M, S) and the last purely sensory stage consists of the responses of Inferotemporal (IT) cortical cells that are tuned to narrow bands of color. Single colors can be decoded successfully using a winner-take-all rule on the responses of IT cells (Zaidi et al 2014, Zaidi & Conway, 2019). However, natural objects generically exhibit signature variations in spectral reflectance that observations show are critical to classifying the objects, so at each neural stage the population signals from each object form a manifold. We used the Ennis et al (2018) hyperspectral database of 42 organic fruits and vegetables, tabulated values of L, M, S photoreceptor spectra (CVRL) and the responses of 279 IT cells to 45 uniform color stimuli distributed over a triangle in color space (Conway & Tsao 2018). We tested how well the LMS manifolds for pairs of different fruits and vegetables could be separated by Fisher Discriminant Analysis (FDA). To our surprise, we found that 95% of the pairs could be separated at an accuracy above 75%, suggesting that the manifolds were not intertwined, and reflecting that human photoreceptors may be well evolved to discriminating between color manifolds of different fruits and vegetables. These results raise the question whether IT manifolds are even more separable. To compute responses of the IT cells to arbitrary lights, the measured responses to the original stimuli were used to approximate smooth tuning curves, and then the IT manifolds were computed for the objects. For IT manifolds, FDA can classify 100% of the pairs with 100% accuracy. Increasing dimensionality thus separates manifolds for natural objects, as expected. Does the narrower tuning of IT cells contribute to the better performance? Broadening or narrowing the tuning curves, while keeping peak locations constant, did not affect accuracy, suggesting that while narrower tuning could be better for decoding single colors, tuning width is less critical for distinguishing between manifolds. Broadening the tuning width does reduce the curvature and volume of the manifold, which could help characterize categories for general recognition in the presence of objects from novel categories. Broadening the tuning width makes each cell's response less sparse and the population response to each manifold less sparse, so sparseness doesn't affect classification accuracy. The high dimensionality of IT population responses thus helps successfully distinguish between the complete sets of surface colors of pairs of fruits and vegetables, while the tuning width may affect categorization efficiency.

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Poster

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Title: Layer-specific dynamics of v1 encoding underlying luminance illusion in awake macaque monkeys

Authors: *T. ZHOU[1], Y. YANG[2], T. WANG[1], Y. WU[3], L. YANG[1], W. DAI[4], X. SUN[1], D. XING[1];
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Abstract: How early visual cortex encodes brightness illusions remains an open question, particularly in terms of the laminar-specific dynamics underlying perceptual luminance. Existing studies have demonstrated that neurons in visual cortex (V1 or V2) can be modulated by brightness illusions, even though such effects are not observed in the retina or lateral geniculate nucleus (LGN). Several circuit-level mechanisms have been proposed to explain illusory brightness perception, including feedforward integration of retinal inputs, horizontal intracortical interactions within V1, and feedback from higher-order areas such as V2 or V4. However, previous findings have largely relied on single-unit recordings, offering limited insight into how brightness information is dynamically processed at the circuitry level and across cortical layers. To investigate these candidate mechanisms, we recorded neural activity across layers of primary visual cortex (V1) in three awake male rhesus macaques (Macaca mulatta) using 24-channel linear array electrodes. Monkeys passively viewed two classes of stimuli: (1) uniform squares of varying physical luminance (real brightness), and (2) same gray squares over varying luminance backgrounds, which induce strong contrast illusions (illusory brightness). While real brightness stimuli elicited robust early responses (~30ms after stimulus onset) across V1 layers, illusory brightness did not evoke fast initial activity. Instead, a delayed and prolonged response emerged around 100ms after stimulus onset, predominantly in the input and deep layers. Luminance tuning curves computed from the late time window revealed sharper selectivity under the illusory condition than during the early phase of the real brightness condition, suggesting temporal evolution and potential cortical refinement of perceptual encoding. Preliminary observations from V2 suggest consistent response dynamics that may strengthen the observed V1 modulation. These results are consistent with a model in which feedback from higher-level visual cortex to subcortical structures, such as the LGN, which subsequently modulates V1 activity in a feedforward way to generate perceptual brightness signals.

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Poster

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Title: Equiluminant Border Ownership Cells as a Missing Link in Color Form Perception

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Abstract: Edges in an image are formed by contrast in either luminance or color. Luminance edges are often seen as the major contributor to form perception. However, equiluminant edges defined purely by color contrast are not rarer than luminance ones [1] and can make borders of figures in natural scenes. Evidence of shape-selective equiluminant cells in V1 and V4 [2, 3, 4] indicate the existence of a color-form subsystem that can represent shapes defined solely by equiluminant borders. Yet, how this subsystem transforms equiluminant orientation to uniform color figure encodings is unknown. We propose the equiluminant version of border ownership (BO) cells, EBO, as the missing links. BO cells encoding the object side of an occluding border were proposed as early signals enabling form perception [5]. With color selectivity in more than 40% of BO cells [5, 6], we propose a subpopulation of these cells signal ownership along equiluminant edges. We suggest a hierarchical computational model providing a mechanistic account of EBO as an early signal for color form perception. Our model extends a previous network [7] that performs BO assignment based on early recurrence from MT cells and lateral modulations in the ventral stream. The proposed extension includes additional mechanisms for color form coding inspired by double-opponent cells in V1 [2]. Dorsal modulation of EBO cells is supported by evidence of contextual modulation in V1 [8] and MT cell responses [9, 10] to equiluminant stimuli. We created chromatic-achromatic stimulus sets and examined EBO and luminance BO cell responses in our model. Like [2, 4], we found no distinct differences in orientation or BO signals in model cells. But EBO cell responses were multiple times stronger to chromatic than achromatic stimuli. Interestingly, while EBO cells respond to both luminance and equiluminant borders, luminance BO cells are blind to equiluminant borders. These results highlight the crucial role of EBO cells in color form perception in presence of equiluminant borders in natural scenes.

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[10] Conway (2014). *Visual neuroscience*, *31*(2), 197-209

Disclosures: P. Mehrani: None. **J.K. Tsotsos:** None.

Poster

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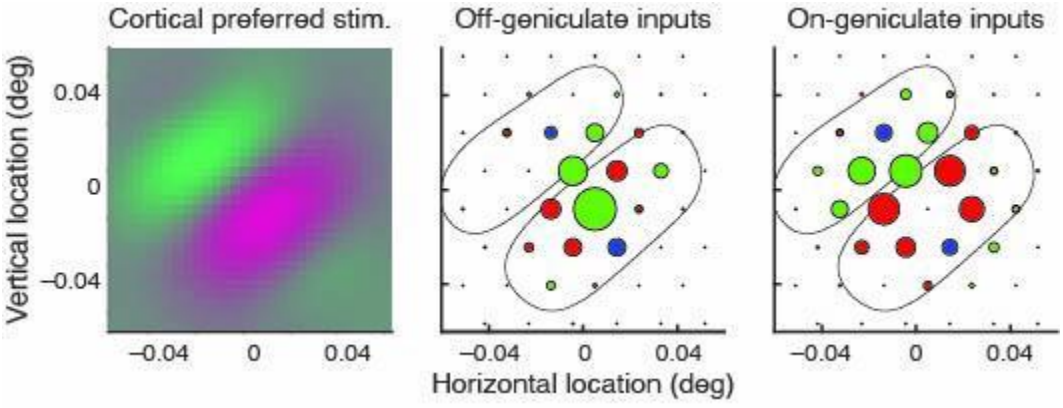
Program #/Poster #: PSTR073.11/K10

Topic: E.06. Vision

Title: A model for the development of double-opponent neurons in macaque primary visual cortex

Authors: M. A. SOMARATNA[1], *A. W. FREEMAN[2];
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Abstract: *Aim.* It has become increasingly clear that the processing of spatial form and chromatic patterns are intertwined in primary visual cortex; double-opponent (DO) neurons exemplify this multiplexing. Our aim was to explore the circuitry underlying DO responses, and its development, using a computational approach. *Methods.* We modelled the macaque midget/parvocellular pathway using five subcortical cell types - cone, horizontal, bipolar, ganglion, geniculate - plus excitatory and inhibitory neurons in the input layer of primary visual cortex. Model parameters were determined from published data. Each cell was modelled with a differential equation and all equations were solved simultaneously to obtain neuronal time courses. For the eccentricity studied, 1°, we assumed that each ganglion cell (and hence, geniculate cell) was driven by a single cone. Geniculocortical synapses were assumed to be plastic, with Hebbian development in two phases: prior to vision, stimuli were achromatic drifting gratings simulating retinal waves; following vision onset, stimuli consisted of drifting equiluminance gratings and pulsed natural images. *Results.* Following development, a substantial number of neurons had DO characteristics. An example is shown in the figure. The preferred stimulus, at left, was calculated by cross-correlating a range of stimuli with the responses they evoked. The receptive field therefore comprises L- and M-cone-specific subfields. The two maps at right show the geniculate inputs driving the cortical cell: each dot represents a geniculate cell, dot diameter gives synaptic strength, and dot colour indicates the cone type driving the geniculate cell. The lower right subfield is dominated by on-centre L (+L) and off-centre M (-M) inputs, and the other subfield by -L and +M inputs, as expected of a DO cell. *Discussion.* According to the model, the circuitry underlying DO cells includes a private line from cones to geniculate cells, unsupervised learning at the geniculocortical synapse, and two stages of development.



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Poster

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JST SPRING JPMJSP2104

Title: Modeling subjective color perception using deep neural networks implementing predictive coding

Authors: *K. UEDA[1], E. WATANABE[2];
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Abstract: Our visual experience is filled with various colors. "Subjective colors" are illusory phenomena in which chromatic colors are perceived from achromatic visual stimuli. Benham's top illusion is a well-known example of subjective colors. This illusion produces color perception when a figure composed only of black and white is rotated. It is known that different color perceptions appear on the inner and outer lines, and that the colors seen on the inner and outer lines switch depending on the direction of rotation. Since such illusory perception is a subjective experience, it is difficult to obtain detailed quantitative data, and individual differences are large. Furthermore, studying the influence of visual learning processes is extremely challenging. In this study, we address these challenges by utilizing AI as an information processing model of the human brain. AI models based on neural circuits are known

to process information similarly to the human brain. The AI we use in this study is called "PredNet." This AI model loads videos and generates predicted future images, implementing the principle of "Predictive Coding." "Predictive Coding" is a hypothesis that the cerebral cortex constantly makes predictions and learns to minimize the prediction error between predictions and actual sensory inputs. Due to this characteristics, PredNet is known to exhibit human-like responses to several visual illusions. We trained PredNet on CG videos of people walking and had it predict the next frame while rotating Benham's top illusion. As a result, PredNet showed color perception similar to humans. We also found that different colors appeared on the inner and outer lines, and these switched when the rotation direction was changed. Furthermore, by using videos with simplified color composition for training, we discovered that the color characteristics of Benham's top illusion may be influenced by moving objects and their opponent colors. From these results, we obtained important insights into the previously unexplained causes of Benham's top illusion by treating AI as a model of the human brain. We would like to engage in discussions while focusing on the similarities and differences in information processing between AI and humans.

Disclosures: K. Ueda: None. **E. Watanabe:** None.

Poster

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Title: System-level computations underlie visual field heterogeneity in human vision

Authors: *S. XUE, A. BARBOT, Q. CHEN, M. CARRASCO;
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Abstract: [Background] Human visual performance varies systematically throughout the visual field, declining with eccentricity and varying around polar angle. Retinal and cortical factors contribute to these asymmetries. However, they only partially account for these perceptual asymmetries. Here we assessed whether and how system-level computations—gain, internal noise, and nonlinearity—account for visual field heterogeneities.
[Method] Using the equivalent noise method and Perceptual Template Model, we estimated these computational properties across multiple eccentricities and cardinal meridians for an orientation discrimination task. At each location, observers discriminated the orientation (±45º off the vertical axis) of Gabor patches (4 and 6 cpd) embedded in varying contrast levels of dynamic white noise. The signal Gabor could appear at one of nine locations: fovea and two non-

foveal eccentricities (parafovea: 4°, perifovea: 8°) x four polar angles (left and right horizontal meridian, upper and lower vertical meridian). We mapped contrast thresholds as a function of noise contrast. Then we fitted Perceptual Template Model to the data to estimate gain, internal noise, and nonlinearity.
[Results] Both gain and internal noise varied with eccentricity, with gain decreasing and internal noise increasing toward the periphery. But only gain varied with polar angle, it was higher along the horizontal than vertical meridian and higher in the lower than upper visual field. Importantly, observers who exhibited a more pronounced eccentricity effect between fovea and 8º eccentricity also show a larger decrease in gain between those eccentricities. These patterns were more pronounced for 6 than 4 cpd.
[Conclusion] Our findings reveal that distinct system-level computations underlie visual field heterogeneities: whereas the eccentricity effect stems from variation in both gain and internal noise, polar angle asymmetries only stem from variation in gain. These results link the perceptual visual field heterogeneity to system-level computations. By uncovering how -in addition to retinal and cortical factors- fundamental computations shape visual performance, this research can inform the design of experiments, interfaces, and environments that align with human vision.

Disclosures: S. Xue: None. **A. Barbot:** None. **Q. Chen:** None. **M. Carrasco:** None.

Poster

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Topic: E.06. Vision

Title: A meta-analysis of afterimages (AIs): their neural substrates and their role as short-term memory in visual neural computation

Authors: *C. Q. WU;
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Abstract: AIs are seemingly simple yet very intriguing visual phenomena. Presently, all the textbooks in vision science, perceptual psychology, and sensory neuroscience introduce these phenomena; meanwhile they also ubiquitously subscribe to two misconceptions: 1. AIs are due to some peripheral adaptation mechanisms occurring in the retina of the eye; 2. +&-AIs are due to different causes. Regarding the first misconception, the contrasting view is that AIs originate in the brain: This view is not new at all, but only recently a multitude of evidence has accumulated pointing to its truthfulness. Two recent critical lines of advances related to afterimages are as follows: 1. LeVay et al. (1985) discovered a representation of the physiological blind spot in Layer 4 of the cortical area V1 (hereafter, V1-L4) in the macaque monkey's brain, and Adams et al. (2007) discovered the same in the human brain; 2. Wu (2011) rediscovered the phenomenon of an observer seeing their own blind spots and correlated this phenomenon with the above neuroanatomical findings. Together, these advances conclusively

pinpoint the neural substrate for the color-specific part of AIs to V1-L4. Concerning the second misconception, Franklin (1765) observed the effect that +&-AIs can be converted into one another as per background illumination. McDougall (1901) rediscovered the Franklin effect and posited that +&-AIs are both due to some chemical or neural persistence (see Figure 1). Based on these results, here I further establish the following claims: 1. AIs should be viewed as a form of short-term memory (STM) in the brain—instead of as peripheral adaptation in the retina; 2. In terms of the neural computational architecture of any cortical area, STM is sandwiched between a feedforward neural network and a feedback counterpart: This neuroanatomical feature allows STM to play the computational role of variable binding. In color vision, Newton (1704) suggested that +AIs serve as STM in successive color compounding which can be conceived as a special case of binding: The view here is a generalization of Newton's this notion.

Table 1. Sources of investigations related to the Retinal View and the Brain View concerning the locus of afterimages in the human visual system.

Investigators	Phenomena / arguments / results	Retinal View	Brain View
Newton (1691)	Newton observed interocular transfer of afterimages and suggested: "It [afterimage] seems rather to consist in a disposition of the sensorium [the part of the brain for sensation] to move the imagination strongly" (p. 154).		✓
Darwin (1786)	Based on an analogy between the retina and the muscular system, Darwin suggested that afterimages are due to retinal adaptation.	✓	
Binet (1886)	Afterimage may exhibit interocular transfer (pp. 43-45).		✓
Delabarre (1889)	There are interocular differences of seeing the same afterimage.	✓	
Craik (1940)	Retinal anoxia ("blinding" an eye by finger-pressing it) disrupts afterimage.	✓	
Weiskrantz (1950)	Afterimage may be centrally produced from mental imagery.		✓
Urist (1958)	When pushing an eye's ball, the eye's scene view shifts in position but its afterimage stays.		✓
Loomis (1972)	Afterimages from long-duration light stimulation with little bleaching are correlated with visual appearance.		✓
Shimojo et al. (2001)	Filling-in visual surface may generate afterimage.		✓
Tsuchiya and Koch (2005)	There is interocular influence on afterimage formation.		✓
Shevell et al. (2008)	Interocular misbinding of color and form may occur.		✓
Zaidi et al. (2012)	Relevant physiological recordings were conducted in the monkey's brain.	✓	✓
Dong et al. (2017)	The Breese (1899) effect: binocular rivalry between two eyes' afterimages is slower than that with physical stimuli.	✓	✓
Kronemer et al. (2024)	Shared mechanisms between afterimages and visual imagery are found.		✓
Wu (2024)	Buffon-McDougall phenomenon: binocular afterimages generated during free-fusion viewing are binocularly coupled even after the two eyes return to their normal convergence.		✓
Wu (2025)	The La Hire phenomenon (i.e., seeing the physiological blind spots as afterimages) pinpoints such afterimages to the neural substrate V1-L4.		✓
Kittikiatkumjorn et al. (2025)	Afterimage color is factored by color constancy.		✓

Table 2. Sources of investigations related to Mariotte's blind spot (BS) and positive & negative afterimages (+&-AIs)

Investigators	Phenomena / arguments / results	Subject
Mariotte (1668)	Discovery of the physiology blind spot in the human eye.	BS
La Hire (1694)	The La Hire phenomenon: an observer seeing their own blind spots and retinal blood vessels.	BS
Charpentier (1898)	An aspect of the La Hire phenomenon: The blind spot may appear as an afterimage to the viewer.	BS
Hubel and Wiesel (1977)	Discovery of ocular dominance columns in V1-L4 (the principal thalamic recipient layer in the primary visual cortex / cortical area V1) of the macaque monkey brain.	BS
LeVay et al. (1985)	Discovery of a cortical representation of the blind spot in V1-L4 of the macaque monkey brain.	BS
Adams et al. (2007)	Discovery of a cortical representation of the blind spot in V1-L4 of the human brain.	BS
Wu (2011)	Correlation between the La Hire phenomenon and the blind spot representation in V1-L4.	BS
Franklin (1765)	The Franklin effect: positive and negative afterimages can be converted into one another as per the viewing illumination of the afterimages.	+&-AIs
McDougall (1901)	Positive and negative afterimages are due to the same chemical (substance X) / neural persistence process in the retina.	+&-AIs

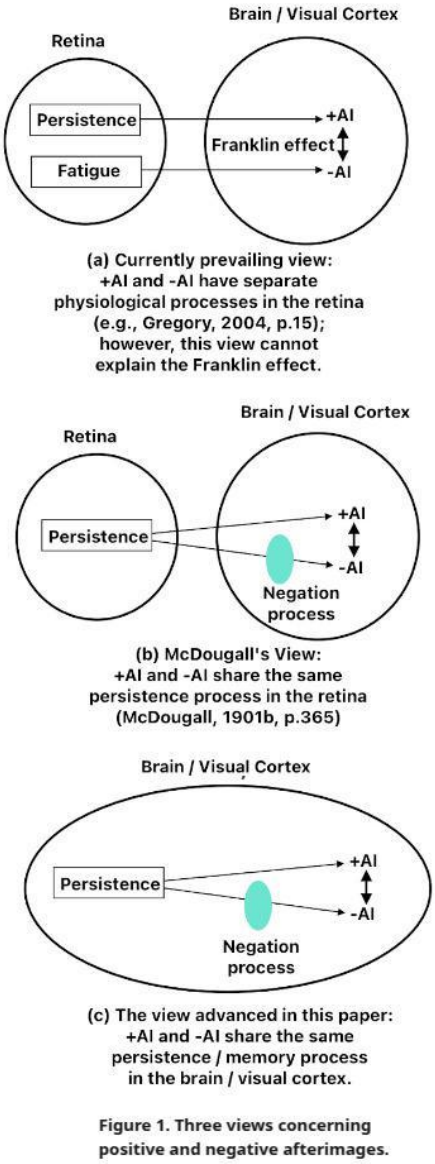


Figure 1. Three views concerning positive and negative afterimages.

Disclosures: C.Q. Wu: None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

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Title: Phase-dependent suppressive perception of visual stimuli synthesized from non-encoding subspace neural activity

Authors: *T. KUWAYAMA[1], G. SHEN[3], N. HAYASHI[1], G. GANESH[4], Y. MIYAWAKI[1,2]; [1]Grad. Sch. of Informatics and Engin., [2]Ctr. for Neurosci. and Biomed. Engin., The Univ. of Electro-Communications, Tokyo, Japan; [3]Inst. for Quantum Life Sci., Natl. Inst. for Quantum Sci. and Technol., Chiba, Japan; [4]Lab. d'Informatique, de Robotique et de Microélectronique de Montpellier (LIRMM), Univ. Montpellier, CNRS, Montpellier, France

Abstract: Visual images are encoded by neural activity patterns in the brain to generate our conscious perception. Although possible neural activity patterns are enormous, they do not necessarily contribute equally to encode visual images that we observe in daily life. We then hypothesize that some of neural activity patterns might be unused and constitute subspaces distinct from those associated with representation of visual information in the real world. To investigate visual perception corresponding to these hypothetical unused, non-encoding subspaces, our previous study defined multiple subspaces based on the explained variance of the early layer of a deep convolutional neural network (DCNN) that models human visual cortex, and examined the contrast detection threshold of human observers for images synthesized by the DCNN that selectively activated the specified subspace. The results showed that images activating the encoding subspace (defined by 99% explained variance) had lower contrast detection thresholds than their frequency-matched but phase-scrambled versions, whereas those activating the non-encoding subspace (defined by 1% explained variance) had higher thresholds. Moreover, when image phase was replaced with that sampled from the non-encoding subspace, the contrast detection threshold increased relative to its frequency-matched, phase-scrambled versions, suggesting that phase characteristics corresponding to the non-encoding subspace might reduce perceptual visibility. In this study, we further examined whether phase characteristics is crucial to modulate the contrast detection threshold, using images whose phase information is sampled from the encoding subspace. Results showed that the contrast detection threshold of the images whose phase information was replaced with that of encoding subspace was lower than their frequency-matched, phase-scrambled versions, unlike the previous results using non-encoding subspace. This finding indicates that contrast detectability can be modulated in opposite directions depending on whether phase information of images is sampled from the encoding or the non-encoding subspace. These evidence suggest that visual images with specific phase information, independent of spatial frequency, might evoke neural activity patterns that fall into the non-encoding subspace, leading to suppressive perception.

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Poster

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Title: Transcranial random noise stimulation modulates contrast sensitivity and polar angle asymmetries across the visual field

Authors: *S. UNER[1], I. AKDOGAN[1], H. KAFALIGONUL[2];
[1]Neurosci., Bilkent Univ., Ankara, Turkey; [2]Neurosci. and Neurotechnology Ctr. of Excellence (NÖROM), Neurosci. and Neurotechnology Ctr. of Excellence (NÖROM), Fac. of Medicine, Gazi Univ., Ankara, Turkey

Abstract: Visual perception is shaped not only by the central visual field but also by polar angle asymmetries in the periphery—namely, horizontal-vertical anisotropy (HVA) and vertical meridian asymmetry (VMA)—which lead to superior contrast sensitivity along the horizontal and lower meridians compared to the vertical and upper fields. While these asymmetries reflect the neural architecture of the visual system, their susceptibility to neuromodulation remains unclear. Contrast sensitivity, a core index of visual function, offers a powerful tool to investigate such plasticity: when characterized across spatial frequencies via the contrast sensitivity function (CSF), it provides a detailed profile of visual sensitivity. Here, we used high-frequency transcranial random noise stimulation (hf-tRNS) to investigate whether external noise modulates contrast sensitivity across the visual field. By examining its effects both at the central visual field and at isoeccentric peripheral locations, we aimed to uncover how neuromodulation interacts with the visual system's intrinsic spatial structure and asymmetries. Participants (n = 22) completed a two-alternative forced-choice orientation discrimination task under both baseline and 1.5 mA tRNS conditions across five visual field locations (center, left, right, upper, lower). We estimated CSFs using a Bayesian adaptive procedure, decomposed into five parameters: peak sensitivity (CS_{max}), peak spatial frequency (SF_{peak}), bandwidth, low-frequency truncation, and area under the CSF (AUCSF). tRNS significantly increased CS_{max} at the center and lower fields, reducing HVA and enhancing VMA. Center bandwidth was reduced under stimulation, suggesting sharper spatial tuning. SF_{peak} remained high at the central field but decreased in upper and left fields, indicating location-specific shifts in spatial resolution. Our findings demonstrate that tRNS modulates contrast sensitivity in a spatially specific manner, enhancing performance in central and lower fields while altering intrinsic asymmetries. These results provide the first evidence that non-invasive electrical brain stimulation can reshape well-established polar angle asymmetries of the visual system, revealing functional plasticity across the visual field. Beyond advancing our understanding of sensory plasticity, these findings also pave the way for developing neuromodulation-based interventions aimed at improving visual function.

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Title: Interocular suppression across binocular rivalry, identical, and disparity tasks: insights from an SSVEP study

Authors: X. SHANG[1], W. HUANG[2], ***C. HAN**[1];
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Abstract: The visual system typically integrates information from both eyes to create a single, stable visual perception. Balanced interocular suppression is essential for normal binocular vision, ensuring a balance of input during the formation of binocular disparity and maintaining luminance constancy. Ongoing debates center on whether the mechanisms of interocular suppression are consistent across different binocular visual tasks—specifically, whether suppression occurs solely between different orientation channels or is universally present regardless of visual stimulus form. In this study, we recorded the strength of interocular suppression across three binocular visual paradigms using steady-state visual evoked potentials (SSVEP). The paradigms included binocular rivalry, where orthogonal gratings were presented to each eye; binocular identical, where both eyes received gratings of the same orientation; and binocular disparity, using stereo random dots to facilitate the integration of input from both eyes. We employed two modulation methods to induce varying intensities of interocular suppression in two experiments. In Experiment 1, we manipulated interocular contrast difference to control the suppression strength; in Experiment 2, we adjusted the relative strength of boundary contours (Han et al., 2020). The results revealed that: (1) interocular differences reflected in the eye-tagging SSVEP signals were correlated with the suppression strength induced by stimulus intensities; (2) no significant differences were found among the three binocular paradigms with respect to inerocular suppression strength, as indicated by the SSVEP signals; and (3) the intermodulation components of the SSVEP signals in the binocular disparity task were significantly higher than those in the other two tasks. These findings suggest that: (a) SSVEP is an effective method for objectively measuring interocular suppression strength, as it does not rely on subjective participant reports; and (b) the lack of significant differences in interocular suppression strength across binocular tasks indicates that early interocular suppression is not specific to orientation channels.

Disclosures: X. Shang: None. **W. Huang:** None. **C. Han:** None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR073.18/K16

Topic: E.06. Vision

Support: NIH Grant EY 30-EY026877 and R01-EY030361-01
Gilbert Family Foundation
Research to Prevent Blindness

Title: Contrast change rather than luminance change dominates a probe-on-pedestal SSVEP

Authors: Z. WU[1], M. T. SCOTT[2], J. L. GOLDBERG[3], *A. M. NORCIA[4];
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Abstract: Psychophysical methods have long been used to study visual threshold sensitivity to small probe elements presented on larger backgrounds (Westheimer, 1965, 1967). Within this framework, temporal sawtooth modulation of the probe creates dynamic changes in both luminance (increments and decrements) as well as changes in contrast (pattern Onsets and Offsets; Sinai et al., 1999). Here we used Steady-State Visual Evoked Potentials (SSVEPs) to probe the relative contribution of local luminance and contrast mechanisms operating well above perceptual threshold. SSVEPs were recorded in response to sawtooth probe-on-pedestal stimuli comprising an array of small hexagonal luminance-modulated probes presented on larger hexagonal static luminance pedestals. Luminance increments and decrements of the probe, defined by the direction of the fast phase of the sawtooth, were presented either above or below the luminance of the pedestal. This paradigm produces fast contrast Onsets (increments presented rising above the pedestal luminance and decrements presented falling below the pedestal luminance) and fast contrast Offsets (e.g., increments starting below and decrements starting above and in either case returning to the pedestal luminance). All stimuli were presented at a fixed 40% Weber contrast defined by the change in probe luminance divided by the background luminance. Stimuli were presented in the upper and lower visual fields (UVF & LVF, respectively) at distinct temporal frequencies allowing visual-field-specific analysis via spectral decomposition. Data were collected from 35 healthy adult participants (11 female). Contrast Onset and Offset responses were distinct from each other, but similar for both luminance-change directions, indicating that the cortical response was dominated by contrast change rather than luminance change. In the UVF, responses were temporally low-pass and Onset responses were larger than Offset responses. In LVF, responses were temporally bandpass and Offset responses were larger than Onset responses. Overall, contrast changes for both Onset and Offset below the pedestal (dark contrasts) evoked larger responses than changes above the pedestal (bright contrasts), especially in the lower visual field. These results indicate that the

cortical response is dominated by the direction of contrast change rather than the direction of luminance change, favoring dark over bright contrast.

Disclosures: Z. Wu: None. **M.T. Scott:** None. **J.L. Goldberg:** None. **A.M. Norcia:** None.

Poster

PSTR073: Visual Processing of Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR073.19/L1

Topic: E.06. Vision

Support: NIH Grant EY029997
NIH Grant EY027023
NIH Grant EY018839

Title: A general relationship between shape, texture and chromatic encoding in the mid-level visual representation in deep convolutional neural networks

Authors: *S. SINHA, W. BAIR;
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Abstract: Previously, we noted a relationship between fill-outline invariance (FOI) and the selectivity for shape vs. texture and for achromatic vs. chromatic stimuli in artificial units in the middle layers of the deep convolutional neural network (CNN) known as AlexNet. This suggested that an axis of representation may arise during training where shape and surface properties are differentially encoded, resembling physiological findings in the primate visual cortex that suggest separate specializations for the encoding of shape and texture in area V4. To determine the generality of this relationship across CNN architectures, we have now extended our analysis to other deep CNNs trained for object recognition, including VGG, ResNet, DenseNet and ConvNeXt. We adopted a visual neurophysiologist's approach by probing networks with stimulus sets previously used to study neurons in macaque V4 (Kim et al., 2019, J Neurosci). Stimuli consisted of simple two-dimensional shapes presented in four conditions: filled vs. outline, and ON (white-on-black) vs. OFF (black-on-white) polarity. We measured FOI by computing correlations between unit responses to filled and outline shapes. We also computed a texture index using the stimuli of Kim et al., and we computed a chromatic sensitivity index by measuring the change in unit response as the color dimensions of natural images were permuted. We found that all CNNs tested showed very high FOI across layers, higher on average than observed in V4 (Popovkina et al., 2019, JNeurophys), and we found that there was a negative correlation between FOI and chromatic sensitivity that grew stronger going from early to middle layers and subsided or vanished in deeper layers. In addition, all networks showed a negative correlation between FOI and texture index in the middle layers that often weakened in deeper layers. We observed several common trends in changes in FOI, chromatic index and texture index across layers. For example, early layers have a broad, sometimes bimodal, distribution of

chromatic index that contracts substantially toward middle values in deeper layers, and texture index often decreases in middle layers before increasing in deeper layers. But some striking differences appeared: ResNet in particular showed abrupt changes from layer to layer in the chromatic index and its relationship to FOI. Overall, we found consistent trends in the relationships between shape and surface properties in the single-unit mid-level visual encoding in CNNs, suggesting a general organizational principle analogous to the functional specialization for surface and boundary features observed in the primate visual cortex.

Disclosures: S. Sinha: None. **W. Bair:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.01/L2

Topic: E.02. Somatosensation – Touch

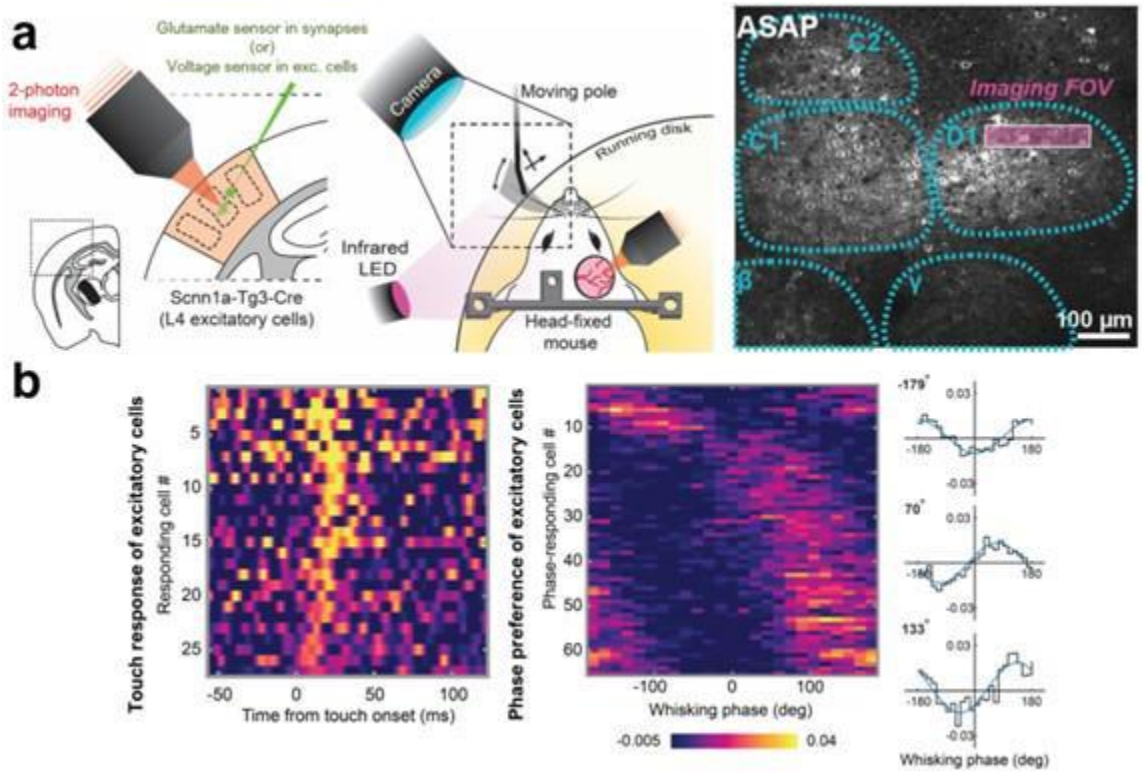
Support: NIH Grant U19 NS107466
NIH Grant U19 NS137920
NIH Grant U24 EB028942
Schmidt Science Fellowship

Title: Local circuitry for processing of mechanical stimuli in layer 4 of the mouse vibrissa cortex

Authors: *R. IYER[1], P. YAO[1], R. LIU[1], D. HANSEL[2], D. GOLOMB[3], D. KLEINFELD[1]; [1]Univ. of California San Diego, La Jolla, CA; [2]CNRS, Paris, France; [3]Ben-Gurion Univ. Negev, Beer-Sheva, Israel

Abstract: Rodent whiskers are active mechanical sensors that are used to actively probe the environment to extract location as well as size, and stiffness of the object. The sensory information, which appears encoded as the self-motion of phase in the whisk cycle, the bending torque upon touch, and the instance of touch, is relayed *via* the thalamus to layer 4 of the vibrissa cortex (vS1-L4), initiating rapid processing. Despite constant self-motion inputs, the excitatory cells in vS1-L4 are remarkably quiescent except immediately following touch (Curtis & Kleinfeld, Nature Neuroscience, 2009; Yu et al. Nature Neuroscience, 2016). The temporal precision of these responses suggests a circuit architecture dominated by strong inhibition and recurrent excitatory connectivity. This study investigates the circuit dynamics of mouse vS1-L4, focusing on the response of the excitatory cells to afferent inputs from the thalamus and local cortical inputs. Using two-photon microscopy with adaptive optics, we capture micrometer-scale synaptic responses using the 4th-generation glutamate sensors (iGluSnFR4f) and cellular voltage changes using 6th-generation ASAP voltage sensors (ASAPv6.3) at >200 Hz in head-fixed freely-whisking mice (Fig. 1a). Preliminary results suggest that, like the thalamocortical projections, vS1-L4 excitatory cells have sub-threshold phase tuning to self-motion and spike selectively upon touch (Fig. 1b). Ongoing experiments assess how response magnitude and

temporal dynamics are modulated by optogenetic suppression of excitatory-to-excitatory and inhibitory-to-excitatory connections. These results advance our understanding toward formulating the vS1-L4 microcircuit and lay the groundwork for a mechanistic model of mechanosensory encoding.



Disclosures: R. Iyer: None. **P. Yao:** None. **R. Liu:** None. **D. Hansel:** None. **D. Golomb:** None. **D. Kleinfeld:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.02/L3

Topic: J.04. Physiological Methods

Support: NINDS Grant RM1NS132981
NEI Grant R01EY035248
NIMH Grant R01MH115900
Ikerbasque Foundation
SECTEI/143/2023

Title: Bidirectional control of cortical activity by chandelier neurons

Authors: *T. ALEJANDRE-GARCIA, R. YUSTE;
Columbia Univ., New York, NY

Abstract: The inhibition-excitation synergy of neuronal ensembles is important for visual perception. Chandelier cells (ChCs) are an anatomically distinct class of interneurons that innervate pyramidal cells at their axon initial segments, where action potentials are generated. This suggests that a single ChC could influence the activity of hundreds of local pyramidal neurons. We are exploring the effects of activating ChCs in the local cortical network, and have found that that, differently from other GABAergic interneurons, ChCs have a dual effect on cortical neurons: they excite them at rest but inhibit them when they are activated. Our results show that ChCs dynamically balance the activity of cortical neurons, preserving a stable operating regime while preserving its dynamical response to visual stimulus.

Disclosures: T. Alejandre-Garcia: None. **R. Yuste:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.03/L4

Topic: I.04. Executive Functions

Support: Foundation for OCD Research

Title: Chemogenetic activation of orbitofrontal cortex impairs action-outcome understanding in marmoset model of OCD: Remediation by mGluR2 positive allosteric modulator

Authors: *S. A. GOULD[1], C. M. WOOD[2], L. MCIVER[3], G. COCKCROFT[3], T. W. ROBBINS[5], A. C. ROBERTS[4];
[2]Dept. of Physiology, Develop. and Neurosci., [3]Physiology, Development, and Neurosci., [4]Physiology, Develop. and Neurosci., [1]Univ. of Cambridge, Cambridge, United Kingdom; [5]Psychology, Univ. Cambridge, Cambridge, United Kingdom

Abstract: Understanding how actions relate to outcomes is crucial for carrying out everyday goal-directed activities. When action-outcome understanding is impaired, it can lead to maladaptive or perseverative actions like those often present in people with obsessive-compulsive disorder (OCD). Human imaging studies have reported heightened orbitofrontal cortex (OFC) Brodmann Area 11 (OFC-11) activity in people with OCD during symptom provocation, as well as altered excitatory:inhibitory balance in the anterior cingulate cortex (ACC). The latter relates to enhanced habitual control in a human contingency degradation test (Biria et al 2023 Nature Comm.). Moreover, causative studies in marmoset demonstrate that activation of OFC-11 or ACC impairs action-outcome understanding in the contingency degradation test (Duan et al 2021 Neuron).
Here, we used a marmoset instrumental contingency degradation test similar to that described by Duan et al (Neuron 2021), as well as anatomical tracing to show connectivity between OFC-11

and ACC. We employed a Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) chemogenetic method to selectively and reversibly activate OFC-11 by systemic injections of DREADDs activator deschloroclozapine (DCZ). We demonstrated that OFC-11 activation impaired marmoset action-outcome understanding on the contingency degradation test where the positive correlation of actions with outcomes is selectively impaired by provision of action-independent ('free') rewards. Marmosets continued responding for a reinforcer despite receiving the same reinforcer for 'free', unlike in control conditions where their responding declined. Furthermore, a systemically administered mGluR2 receptor positive allosteric modulator (AZD8529) dose-dependently antagonised the effects of OFC-11 activation to impair perception of action-outcome contingencies, presumably by its action at presynaptic receptors to reduce glutamate release, whereas the drug did not impact baseline responding. AZD8529 is well tolerated in humans, so may present a novel opportunity to treat action-outcome impairments in people with OCD and related disorders.

Disclosures: S.A. Gould: None. **C.M. Wood:** None. **L. McIver:** None. **T.W. Robbins:** None. **A.C. Roberts:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.04/L5

Topic: E.02. Somatosensation – Touch

Support: SNSF 32003B_207719
SNSF PZ00P3_208996

Title: Mere cortical processing activates somatosensory thalamic and brainstem nuclei after cervical spinal cord injury

Authors: *P. HOWELL[1], F. RABE[1], P. FREUND[2], N. WENDEROTH[1], S. KIKKERT[1]; [1]Swiss Federal Inst. of Technol. (ETH), Zurich, Switzerland; [2]Spinal Cord Injury Ctr. Balgrist, Univ. Hosp. Zurich, Zurich, Switzerland

Abstract: A cervical spinal cord injury (SCI) causes profound sensorimotor disruption, resulting in sensory deprivation and paralysis below the lesion level. However, using attempted hand movements, we previously demonstrated that somatotopic representations in the primary somatosensory cortex (S1) can persist even in the absence of peripheral somatosensory input. This suggests that intracortical processing can sustain activity in S1. Similarly, research in cats and non-human primates has shown that top-down cortical processes can modulate activity in subcortical somatosensory nuclei. However, it remains unknown to what extent top-down processing may activate thalamic and brainstem somatosensory nuclei in humans. Therefore, in this study, we explored sensory processing in cervical SCI patients, who lack or have severely diminished bottom-up somatosensory inputs, as a model to test whether top-down processing can

elicit activation across somatosensory thalamic and brainstem nuclei. We collected 3T fMRI data from 16 chronic cervical SCI patients and 20 age-, sex-, and handedness-matched able-bodied control subjects. Participants were visually cued to make overt or attempted (in case of hand paralysis) right- and left-hand movements. We then analysed hand activity within the ipsi- and contralateral cuneate nuclei of the brainstem, ventral posterior lateral nucleus (VPL) of the thalamus, and S1 hand area. We found a distinct laterality of hand activity in our control participants, with the highest activation observed in the ipsilateral cuneate nucleus, contralateral VPL nucleus, and the contralateral S1 hand cortex. Importantly, we found that this canonical pattern of laterality was preserved in tetraplegic patients, even in participants with a complete SCI; i.e., complete hand paralysis and disconnect between the brain and periphery. Notably, we did not observe any significant correlations between the amount of preserved activity in patients and clinical measures such as retained sensorimotor hand function, time since injury, and the amount of preserved spinal cord fibres. Together, our findings reveal preserved activation of the hand somatosensory relay nuclei in tetraplegic patients despite absent or only partially intact transmission of bottom-up sensory input, extending the notion of preserved representations beyond the cortex. This demonstrates, for the first time, that mere cortical processing can selectively activate the VPL and cuneate nuclei in humans through top-down processing. Importantly, this provides new therapeutic avenues for future interventions aimed at restoring somatosensation following SCI.

Disclosures: P. Howell: None. **F. Rabe:** None. **P. Freund:** None. **N. Wenderoth:** None. **S. Kikkert:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.05/L6

Topic: I.04. Executive Functions

Support: NIH 5T32MH129274-02

Title: An exploratory study of corticostriatal glutamate transmission during compulsive behaviors in mice

Authors: *Z. N. FOKAKIS[1], A. VARGHESE[3], L. A. VOLPICELLI-DALEY[4], J. A. HARDAWAY, III[2];
[2]Psychiatry, [1]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Univ. of Alabama, Birmingham, AL; [4]UAB, Birmingham, AL

Abstract: Obsessive-compulsive symptoms (OCS) are a complex set of behaviors that arise in multiple psychiatric disorders, including obsessive-compulsive disorder (OCD), schizophrenia (SZ), and Tourette's syndrome. These symptoms also arise in neurodegenerative disorders including Parkinson's disease and Frontotemporal dementia. OCS are characterized by

dysfunction in corticostriatal circuits. Imaging studies of OCD patients demonstrate hyperconnectivity of the ventromedial prefrontal cortex to cortical and subcortical regions such as the motor cortex, striatum, and thalamus, among others. Knockout studies have demonstrated that disruptions of excitatory synapse activity within the striatum lead to increases in compulsive behaviors. Additionally, electrophysiology studies have shown that pathologic striatal activity in an OCD mouse model is related to upregulated M2 inputs and not to intrinsic excitability of the striatal neurons. However, the direct role of glutamate in this pathway has yet to be defined. In this exploratory study we use the fluorescent biosensor iGluSnFR3.v857 to observe corticostriatal glutamatergic neuronal function *in vivo* in mice during serial order tasks which allow the comparison of sequential or perseverative actions. We expressed iGluSnFR3 bilaterally in the dorsomedial striatum (DMS) of 8-10 week old male and female C57BL/6J mice (N=6), followed by placement of indwelling chronic optical fibers at the iGluSnFR3 striatal injection sites. In a separate cohort of C57BL/6J mice (N=3), AAVrg-hSyn-Cre.WPRE was injected bilaterally in the DMS and FLEX-JGCaMP8f injected in M2 with placement of indwelling chronic optical fibers at the GCaMP8f injection sites. Mice were trained in their home cages for two weeks on a serial order task. We then recorded optical signals from the DMS while mice underwent the serial order assay, open field test, and a self-initiated running wheel assay at 4-, 8-, 10-, and 12-weeks post-injection. Data were analyzed by researchers blinded to the conditions of the trial. Preliminary results suggest reduced glutamate transmission in the DMS, as well as reduced activity of M2-DMS neurons during perseverative errors within the serial order task.

Disclosures: Z.N. Fokakis: None. **A. Varghese:** None. **L.A. Volpicelli-Daley:** None. **J.A. Hardaway:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.06/L7

Topic: E.02. Somatosensation – Touch

Support: NIH Grant P41 EB018783 (Wolpaw)
NYS Spinal Cord Injury Research Board C37714GG (Gupta)
NYS SCIRB C38338GG (Wolpaw)
Stratton Veterans Affairs Medical Center
Veterans Affairs RRD I21 RX004410

Title: Development of a portable cortical evoked potential operant conditioning system

Authors: *D. GUPTA[1,2], J. HILL[3,2];
[1]Natl. Ctr. for Adaptive Neurotechnologies, Stratton VA Med. Center, Albany, NY, Albany, NY; [2]Electrical and Computer Engin., Univ. at Albany, State Univ. of New York, Albany, NY; [3]Natl. Ctr. for Adaptive Neurotechnologies, Albany Stratton VA Med. Ctr., Albany, NY

Abstract: Objective**:** The open-source Evoked Potential Operant Conditioning System (EPOCS) platform has been instrumental in advancing translational targeted neuroplasticity rehabilitation research, such as H-reflex-based operant conditioning for spinal cord injury (Hill et al., 2022, Thompson et al., 2013). EPOCS was originally developed for real-time feedback of electromyography (EMG)-based responses—such as the H-reflex and motor evoked potentials—using a 1-2 channel electrode configuration. These EMG responses typically exhibit high signal-to-noise ratio (SNR). We have now customized EPOCS to support neural evoked responses to extend its applicability to brain signal based conditioning. Method: Cortical-EPOCS (C-EPOCS) can now be used with a full-head multichannel electroencephalography (EEG). Given EEG's lower SNR, we added a modular signal processing layer with spatial and temporal filters for real-time denoising and signal enhancement. The system continues to monitor peripheral responses such as compound muscle action potentials and H-reflexes, now with added monitoring of sensory nerve responses to maintain consistent afferent excitation across sessions. The stimulation protocol is adapted to be contingent on background brain activity being in a predefined state or at rest, as an alternative to muscle contraction. We demonstrate this framework for real-time feedback of somatosensory evoked potentials (SEPs) elicited by tibial nerve stimulation. A streamlined configuration was also tested in 5 participants, where the EEG and EMG are acquired via a single headset and computer—improving portability, reducing hardware cost, and simplifying setup. Results: We demonstrate real-time single-trial SEP N70 extraction using C-EPOCS in two participants, and portable C-EPOCS in five participants. Data were collected with informed consent (VAMC IRB #1584762). A recruitment module was used to acquire and process recruitment curve data; the analysis module rapidly visualized SEP N70 magnitude across training trials; the training module delivered real-time SEP feedback. Background EEG was continuously displayed via gauges. Realtime single-trial SEP were clearly detectable in 76 $\pm$ 0.13 % trials. Significance: The portable C-EPOCS system—with reduced equipment footprint and rapid setup—supports neuroplasticity-driven rehabilitation R&D, particularly in sub-acute recovery settings where patient endurance and attention are limited.

Disclosures: D. Gupta: None. **J. Hill:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.07/L8

Topic: J.04. Physiological Methods

Support: Air Force Office of Scientific Research Award FA9550-21-1-0401

Title: Novel Optical Parallelized Diffuse Correlation Spectroscopy Distinguishes Subtle Task-Specific Prefrontal Cortical Activity

Authors: *A. CHEGU[1], L. KREISS[1], P. MCKEE[1], S. A. HUETTEL[2];
[2]Ctr. for Cognitive Neurosci., [1]Duke Univ., Durham, NC

Abstract: Non-invasive optical imaging measures physiological markers of brain activity at high temporal resolution, with fewer restrictions, and at a lower cost than traditional neuroimaging techniques. Here we explore the potential of a new technique, Parallelized Diffuse Correlation Spectroscopy (PDCS), which measures the scattering of near-infrared light using a single-photon avalanche diode (SPAD) array. Fluctuations in the resulting speckle patterns create a calculated blood flow index (BFI) that provides insights into capillary perfusion, often underestimated by traditional absorbance-based techniques. Building on prior work distinguishing prefrontal cortex (PFC) blood flow in resting vs. task-activated states, this study evaluates PDCS' ability to differentiate PFC activation across executive functioning tasks. Participants completed a Go-No-Go task (targeting the right inferior frontal cortex [RIFC]) and a Verbal Fluency Task (VFT; targeting the left inferior frontal cortex [LIFC]) while PDCS measured regional blood flow. Results were compared to functional near-infrared spectroscopy (fNIRS), a well-established technique that tracks cortical blood oxygenation via light absorption. PDCS data revealed distinct task-specific activation: Go-No-Go increased RIFC activation relative to LIFC and VFT, while VFT increased LIFC activation relative to RIFC and Go-No-Go. These findings demonstrate PDCS' sensitivity to subtle, region-specific neural activity, supporting its potential as an effective tool for optical blood-flow neuroimaging.

Disclosures: A. Chegu: A. Employment/Salary (full or part-time): Duke University. **L. Kreiss:** A. Employment/Salary (full or part-time): Duke University. **P. McKee:** A. Employment/Salary (full or part-time): Duke University. **S.A. Huettel:** A. Employment/Salary (full or part-time): Duke University.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.08/L9

Topic: H.02. Reward and Appetitive Learning and Memory

Title: D2 receptor loss in cortical GABAergic interneurons increases putative glutamatergic neurons in the anterior cingulate cortex and reduces behavioral sensitivity to NMDA receptor antagonism.

Authors: *Y. THABET[1], D. L. GRAHAM[2], G. D. STANWOOD[3];
[1]Biomed. Sci., Florida State Univ., Tallahassee, FL; [2]Biomed. Sci., [3]Col. of Med., Tallahassee, FL

Abstract: Dopamine D2 receptors (D2Rs) have been studied and used as a therapeutic target for the treatment of various neuropsychiatric disorders including schizophrenia. They also play a critical role during neurodevelopment and in the regulation of the excitatory/inhibitory balance, yet their specific effects in the anterior cingulate cortex (ACC), a region known for cognitive, attention, and mood control, are not fully characterized. Here we used a D2R conditional knockout (cKO) mouse model that targets the deletion of D2Rs in medial ganglionic eminence

(MGE)-derived GABAergic interneurons during development. In these mice, we surprisingly observed an increased density of glutamatergic neurons in the adult ACC of D2R-cKO mice (20-30% increase across different cortical layers), identified by *Slc17a7* expression through RNAscope *in situ* hybridization. There was no change in the expression of GABAergic interneurons, marked by *Gad1* expression. To further assess the specificity of this model, we quantified excitatory and inhibitory neuron densities in the CA1 region of the hippocampus and found no significant changes in either marker. These findings support a region-specific, non-cell-autonomous effect of D2R loss on the excitatory neuronal density in the ACC. In addition, GABAergic D2R-cKO mice exhibited significantly blunted locomotor activity to the NMDA receptor antagonist MK-801 compared to the control group (27-35% blunting in locomotion across multiple doses). These effects were observed across multiple doses of MK-801. Ongoing experiments include additional behavioral testing with memantine. Altogether, these results suggest that the early loss of D2Rs in inhibitory neurons can affect the development of cortical excitatory neurons and alter behavioral responses to NMDA receptor antagonists, providing a better understanding of D2R-mediated regulation of developing cortical circuitry and neurobehavioral trajectory.

Disclosures: Y. Thabet: None. **D.L. Graham:** None. **G.D. Stanwood:** None.

Poster

PSTR074: Cortical and Thalamic Dynamics Underlying Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR074.09/L10

Topic: E.02. Somatosensation – Touch

Support: NIH Grant NS16446
Wellcome Trust Senior Research Fellowship 063259/Z/00/Z

Title: Reorganized cortical somatotopy reflects thalamic somatotopy in monkeys with dorsal spinal cord injuries sustained early in development

Authors: *N. JAIN[1,2], H. QI[2], A. RAMAN[3,4], D. C. LYON[5,2], J. H. KAAS[2];
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Abstract: Injuries to the dorsal columns of the spinal cord at cervical levels in adult monkeys result in reorganization of the somatosensory cortex, thalamus and brainstem nuclei such that the intact chin inputs expand into the deafferented hand cortex. We have shown that the details of the reorganized map in area 3b are not an exact reflection of the somatotopy in the ventroposterior lateral nucleus (VPL) (Jain et. al., J Neurosci, 28:11042, 2008). In the deafferented parts of VPL, in addition to the chin, there is also an expansion of the occiput, neck

and shoulder inputs (see Halder et al., Cereb Cortex, 2017, doi: 10.1093/cercor/bhx25600. We have further shown that if the lesions of the dorsal columns of the spinal cord are done between postnatal day (PND) 3 and PND5 the somatotopy in the cortex is nearly normal-like despite the deafferentation (Jain et al., PNAS 122:e2417417122, 2025). However, if the lesions are made between PND9 - PND12 the deafferented cortex remains largely non-responsive to tactile stimulation on any part of the body. There is a limited expansion of the chin and arm inputs in the deafferented cortex. Here we determined the somatotopy in the ventroposterior nucleus (VPN) of the thalamus in four of the monkeys in which somatotopy of area 3b and the adjacent somatosensory cortex was determined. The monkeys had lesions at PND3 (two monkeys), PND5 and PND9. The somatotopy was determined using standard multiunit mapping techniques using tungsten microelectrodes. Our data show that as for area 3b, the somatotopy in the VPL is very normal-like in the monkeys with lesions made at PND3 and PND5 suggesting that alternate somatotopically appropriate inputs reactivate both, the somatosensory cortex as well as VPL. In the monkey lesioned on PND5 a very limited expansion of the chin was observed in the deafferented hand region of both VPL and area 3b. However, in the monkey with lesion at PND9 neurons in the VPL, as in area 3b, were largely non-responsive to peripheral stimulation. Thus, unlike in monkeys with lesions as adults, the somatotopy in the VPL and the somatosensory cortex is similar reflecting a limited capacity of reorganization at PND9 in both VPL nucleus and the cortex.

Disclosures: N. Jain: None. **H. Qi:** None. **A. Raman:** None. **D.C. Lyon:** None. **J.H. Kaas:** None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.01/L11

Topic: F.04. Voluntary Movements

Support: Helen Hay Whitney Foundation
NIH Grant R35NS111643

Title: Neural geometry of forelimb proprioception in the cervical spinal cord

Authors: *T. BOLLU, M. D. GOULDING;
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Abstract: Precise, real-time sensory feedback is essential for skilled movement. While the architecture of sensory feedback pathways is known, their neural code and its construction remains unclear. Here, using high-density in vivo electrophysiology and precise neural manipulations, we identify the neural representation of the forelimb's kinematic state and its construction in the cervical spinal cord of mice. We identify two classes of neurons - neurons that jointly encode speed, position and direction. And neurons that precisely fire at specific limb

positions or in grid-like firing patterns that tile the kinematic space. Population activity was constrained to a low dimensional space that was an ordered representation of the position and speed of the limb. Muscle sensory afferents, but not cutaneous sensory afferents, were required to construct this neural geometry. In mice reaching to spatial targets, transient perturbations of muscle afferents led to end-point errors as predicted by the deficits in the neural code. Our results identify the computations spinal networks perform to generate the percept for forelimb movement.

Disclosures: T. Bollu: None. **M.D. Goulding:** None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.02/L12

Topic: F.04. Voluntary Movements

Support: JST/PRESTO (JPMJPR23S8)

Title: Single-trial motor adaptation dynamically alters perceived hand position

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Abstract: When interacting with the environment, the central nervous system (CNS) receives sensory information used not only to explicitly generate perception but also to implicitly guide motor actions. To maintain coherence within the action-perception loop, the CNS must reconcile the distinct computational principles: perception is often shaped by Bayesian inference (Ernst & Banks, 2002), while motor control can involve divisive normalization (Hayashi et al., 2020; Makino et al., 2024), potentially leading to conflicts between action and perception. Motor adaptation would serve as a compensatory mechanism; prolonged adaptation (e.g., over 100+ trials) has been shown to systematically shift perception (Ostry et al., 2010). However, motor adaptation also occurs on a trial-by-trial basis, raising the possibility that perception may dynamically shift with each motor update. In this study, 24 human participants performed 10-cm right-hand arm movements under clamped feedback, where hand motion was constrained with channels to one of 12 directions from -23° to 23°. Each trial block comprised three successive trials: a reference (no shift, 0°), a shift trial (one of 12 perturbations), and a decision trial where participants judged the shift trial movement (leftward/rightward) relative to the reference. We quantified two types of motor responses: the feedback response, defined as the lateral force exerted against the channel during the shift trial, and the adaptive response, defined as the change in lateral force from the reference trial of the current block to that of the subsequent block. Because the magnitude of these responses varied across trials despite identical perturbations, we categorized trials by the direction of these responses (more leftward or rightward) and examined the corresponding perceptual reports. Strikingly, we found that when

participants exhibited leftward adaptive responses, their perceived hand position shifted leftward—and vice versa ($t = 2.96$, $p = 0.0075$)—with perceptual shifts reaching up to 1.25 mm, approximately 70% of the effect size observed in the extended motor adaptation (Ostry et al., 2010). Notably, this perceptual effect was not observed when we categorized reports by feedback responses ($t = 1.46$, $p = 0.16$). Furthermore, this perceptual warping did not occur when the arm was passively moved ($t = 1.03$, $p = 0.32$). These results demonstrate that even single-trial motor adaptation can significantly and systematically influence perceptual judgments, highlighting the tight and dynamic coupling between action and perception.

Disclosures: T. Hayashi: None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.03/L13

Topic: F.04. Voluntary Movements

Support: Fondazione Cariplo e Fondazione CDP Grant n. 2023-2315

Title: Bridging feeling and motion: insula-premotor dynamics in the processing of action vitality forms

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Abstract: Typically, people perform actions in a valenced - positive or negative - way, depending on their attitudes or desires. These forms of action are named vitality forms (VFs). While it is well established that action goals are mediated by a parieto-frontal network, less is known about the processing of VFs. Recent fMRI studies suggest that the insula—and its connections with the parieto-frontal circuit—plays a crucial role in VFs processing. However, a key question remains: how does our internal affective state shape our motor behavior? To explore this issue, we conducted an fMRI study involving 22 participants. Participants were required to perform two sequential tasks: 1) to evoke either a positive (enthusiastic) or a negative (angry) affective state (feeling task); 2) to execute an action while maintaining these affective states (execution task). Univariate analysis revealed activation in the dorso-central insula (DCI) and prefrontal cortex (PFC) during the feeling phase. During the subsequent action execution, brain activity extended from the DCI and PFC to the premotor (PM) and parietal areas. To determine the directionality of information flow among these nodes - PM, DCI, and PFC - involved in the action VFs processing, we employed Dynamic Causal Modeling. Bayesian model comparison showed that, during the feeling task, affective set originates in INS, which, together with the PFC, modulates the activity of PM. In contrast, during execution, motor commands

emerge from PM and influence activity in the INS and PFC. These findings shed new light on the dynamic neural interplay underlying the expression of VFs. When an action is guided by an internal state, that state is first represented in the DCI, which influence the motor response by modulating the parieto-frontal network, likely helping PM to select the appropriate VFs of the motor pattern. During the execution of affective actions, the PM, in turn, modulates DCI providing feedback about the VFs of the ongoing action. In this way, a motor act acquires its affective colour.

Disclosures: G. Di Cesare: None. **Y. Koush:** None. **A. Sciutti:** None. **K.J. Friston:** None. **G. Rizzolatti:** None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.04/L14

Topic: F.04. Voluntary Movements

Support: NS112367

Title: Effects of constant and random vibration on proprioception and multisensory integration

Authors: *B. S. ISA[1], J. MATTEN[1], R. WATTERSON[1], S. PIERSANTI[1], H. J. BLOCK[2]; [1]Program in Neurosci., [2]Dept. of Kinesiology, Indiana Univ. Bloomington, Bloomington, IN

Abstract: In motor behavior, the integration of visual and proprioceptive cues about hand position plays a significant role in movement accuracy. However, these sensory inputs are subject to noise, which introduces uncertainty in the perception of the body and the environment. The brain is thought to weigh these inputs and rely more on less-noisy modality. Experimentally, visual noise can be easily manipulated, but proprioceptive noise is more complicated. Some have used vibration with mixed results. Here we compared two vibration protocols on the left forearm flexors and extensors to see if they affect proprioception and visuo-proprioceptive integration in estimating the position of the unseen left index finger. Healthy young adults were assigned to receive either constant 80 Hz vibration, or a random protocol where the frequency changed every 40s within the range of 60-100 Hz. Participants were seated in front of a 2D virtual reality double-sided touchscreen apparatus. A custom device consisting of two motor modules of 12mm diameter motors with a 3D printed casing was used to apply vibration. Participants were asked to indicate with their right index finger on the top touchscreen where they perceived a series of proprioceptive targets consisting of the left index finger on the bottom touchscreen. Unimodal visual and bimodal visuo-proprioceptive trials were also included. There were no speed requirements, no direct vision of the hands, and no performance feedback or knowledge of results. Each participant completed three blocks of trials with vibration "OFF" (1st block), vibration "ON" (2nd block), and vibration "OFF" (3rd block). For each set of 20 unimodal proprioceptive trials, we computed the variance of proprioceptive estimates in several different

ways: Lateral and sagittal dimensions, major and minor axes of the confidence ellipse, root mean squared error, and variance of 2D vector magnitudes. Preliminary results in 16 participants (7 constant, 9 random) suggest the random protocol may be more effective than the constant protocol at increasing variance in proprioceptive target estimation. In particular, variance in the dimension of the short axis of the confidence ellipse appears to increase in the presence of random vibration, rising from 75 ± 8 mm^2 to 97 ± 12 mm^2 (mean ± SE). In contrast, variance in this dimension changed from 56 ± 8 mm^2 to 66 ± 10 mm^2 in the presence of constant vibration. These results could also indicate that arm biomechanics may influence the geometry and sensitivity of the brain's high level proprioceptive representation, leading to spatial idiosyncrasies in the brain's estimate of hand position and in the effects of vibration.

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Poster

PSTR075: Reaching Control: Action and Sensation

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Program #/Poster #: PSTR075.05/L15

Topic: F.04. Voluntary Movements

Support: NIH Grant R01HD090125
NIH Grant R01-CRCNS-NS120579
NIH Grant NIH-R37-HD087089

Title: Neural mechanisms of discrete movements are re-used for a continuous stabilization task

Authors: *R. H. CHOWDHURY[1], B. M. DEKLEVA[2], B. M. KARPOWICZ[3], E. R. OBY[4], M. SADEGHI[5], R. SHARIF RAZAVIAN[7], S. BAZZI[8], C. PANDARINATH[9], P. J. LOUGHLIN[1], D. STERNAD[6], A. P. BATISTA[1];
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Abstract: Carnival games contain a variety of movements, from throwing to whack-a-mole, to balancing a pole on your hand. These movements are driven by sensory feedback to different degrees—while throwing is a single, feedforward movement, pole-balancing is interactive and requires feedback-driven adjustments. Motor neuroscience has explored single-movement tasks in great depth, but we understand far less about the motor control of these more continuous, interactive tasks. This work attempts to illuminate this aspect of neural control, examining motor cortical activity as monkeys performed a pole-balancing-like task.

In this task, called the Critical Stability Task (CST), monkeys made hand movements to control the horizontal position of a cursor on a screen. The task began with the cursor in the screen's center. On trial start, the cursor's motion became unstable: the cursor velocity became proportional to the sum of hand and cursor positions. Left alone, the cursor would fly to one side. The monkey counteracted this by moving his hand opposite to the cursor. To be rewarded, the monkey had to keep the cursor within 5 cm of the center for 3 seconds.
Though the CST was continuous, monkeys often made large, discrete movements to stabilize the cursor. For comparison, we interleaved CST trials with an explicitly intermittent movement task, where monkeys made sequential reaches to randomly positioned targets (Random Target Task—RTT). Hand movements in the two tasks were similar to each other but quite different from the single, large movements made during a classic center-out reaching task.
Motor cortical neural population activity covaried similarly in the CST and RTT, occupying nearly the same population subspaces. We extracted a subspace of neural activity during RTT that corresponded to triggering individual movements, regardless of the movement direction or extent. Projecting CST activity into this space revealed a similar structure—this subspace activated prior to the large discrete movements in CST. In a separate subspace, we found that a single neural decoder could accurately predict hand velocity across both tasks (accounting for 95% of variance accounted for by single-task decoders). These similarities suggest that the neural machinery for discrete movements is at least partially re-used for this continuous stabilization task.
To date, motor neuroscience has focused on the neural activity in relatively simple movements. The task we studied offers a window into the neural activity generating interactive behaviors, offering a steppingstone towards understanding the complex tasks we perform every day.

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Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.06/M1

Topic: F.04. Voluntary Movements

Support: ANR
Institut NeuroMarseille
Carnot STAR

Title: Proprioception: some consistent and not so consistent data about its evaluation and link to motor control

Authors: *F. R. SARLEGNA;
Inst. of Movement Sciences, Aix-Marseille Univ. & CNRS, CNRS, Marseille, France

Abstract: It is well established that proprioception is critical for motor control (Rothwell et al. 1982; Ghez & Sainburg 1995; Hermsdorfer & Nowak 2008; Sarlegna & Sainburg 2009; Jayasinghe et al. 2021). A consistent finding is that individuals with severe proprioceptive (and tactile) deficits perform worse than neurotypical individuals when reaching or manipulating objects. However, we currently do not have a consensual definition of proprioception, and also do not have a consensual method to assess proprioception. In neurotypical right-handed individuals, using an ipsilateral passive matching task has consistently revealed that arm proprioception is more precise for the non-preferred arm compared to the (so-called) preferred arm, more specifically at the elbow and the wrist (Abi Chebel et al. 2022, 2023; with preference being defined based on Oldfield 1971 questionnaire). Recently, we asked neurotypical participants to rate their proprioceptive performance in this task for both arms: right-handers reported higher ratings for the dominant arm, while proprioceptive performance was actually better for the non-dominant arm. We also asked blind participants to rate their proprioceptive performance and right-handers reported higher ratings for the dominant arm, while actual proprioceptive performance was not significantly lateralized. Using a similar ipsilateral passive matching task, we found proprioceptive deficits for sensory neuropathy patients (Yousif et al. 2015; Miall et al. 2018). However, these deficits were smaller than expected from i) clinical tests which failed to reveal any tendon reflex, and ii) neurophysiological testing which failed to reveal any somatosensory-evoked potential. Overall, such inconsistencies lead to a new theoretical, multi-layer model of proprioceptive function (Héroux et al. 2022; Abi Chebel et al. submitted) which suggests that multiple tests are necessary to assess all aspects of proprioception. Some of these data on proprioceptive perception can be correlated to data on arm / hand motor control.

Disclosures: F.R. Sarlegna: None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.07/M2

Topic: F.04. Voluntary Movements

Support: R01 NS111479
DP2 NS105555
U19 NS112959
RF1 NS128898
R00 NS088193
F31 NS115477
F32 NS120998

Title: The lateral reticular nucleus is required for the rapid and precise execution of goal-directed forelimb movements

Authors: E. J. SANDERS, **K. HUANG**, M. PECK, B. FRIAS, A. LETELIER, A. BHEGADE, H. GAO, *E. AZIM;
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Abstract: Dexterous forelimb movements require the exquisite coordination of dozens of muscles to achieve a desired goal. The precision of these behaviors is achieved in part through rapid online corrections of motor output throughout the course of a movement. While sensory feedback provides information critical for assessing movement outcomes, delays inherent in sensorimotor pathways necessitate the use of faster predictive control mechanisms to generate online corrections for achieving rapid yet precise movements. The cerebellum is thought to perform computations necessary for these online corrections by using internal copies of motor commands to predict and refine movement outcomes. The lateral reticular nucleus (LRN) is a brainstem nucleus that is a major recipient of internal copy signals from the cervical spinal cord and conveys these signals to the cerebellum through mossy fiber projections. However, little is known about how the LRN contributes to the execution and coordination of forelimb movement. To address these questions, we are: (1) monitoring the activity of LRN circuits during reaching in mice to evaluate the encoding of movement information in the LRN during behavior; and (2) perturbing LRN circuits on multiple timescales to evaluate the impacts on dexterous reaching movements. *In vivo* single-unit recordings of LRN activity during movement execution show that LRN neurons encode kinematic features of ongoing forelimb movements. Additionally, while chronic ablation of LRN neurons has minimal effect on the performance of goal-directed reaching, acute optogenetic inhibition of LRN neuronal activity causes a reduction in movement speed and increased variability in reach trajectories, effects consistent with an increased reliance on delayed sensory feedback. Our findings suggest that the LRN conveys kinematic features to the cerebellum that are used for online predictive control.

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Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.08/M3

Topic: F.04. Voluntary Movements

Support: RAISE - Robotics and AI for Socio-economic Empowerment (ECS00000035)

Title: Proprioceptive Augmented Reality improves temporal accuracy in movement reproduction

Authors: *M. BOVE[1], A. BISIO[1], M. BIGGIO[1], E. SOLDI[2], A. MASSA[3], C. IESTER[1], L. BONZANO[1];
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Engineering, Univ. of Genoa, Italy, Genoa, Italy; [3]Dept. of Informatics, Bioengineering, Robotics and Systems Engineering, Univ. of Genoa, Genoa, Italy, Genoa, Italy

Abstract: Virtual reality (VR) can be a valuable tool in rehabilitation research due to its ability to simulate real-world scenarios in a controlled, immersive environment. Proprioceptive Augmented Reality (PAR) elements could enhance these experiences by enriching proprioceptive feedback and influencing motor execution and perception. Action observation combined with proprioceptive stimulation (e.g., 80Hz - muscle vibration inducing muscle spindle activation) has been shown to increase plasticity in the primary motor cortex. At the same time, it activates a frontoparietal network whose activity is associated with the capacity of the single subject to develop plasticity, suggesting its potential for motor learning. The possibility to learn by imitating is mediated by the possibility to correctly reproduce the observed movement. In this study, we investigated the ability of healthy participants to replicate the features of the movements experienced via a standard VR protocol and a PAR protocol. Twenty-three healthy participants were divided into two groups based on the stimulation type: the VR group only observed repetitive flexion-extension movements at the elbow and wrist joints of a virtual arm in first-person perspective in an immersive VR environment, while the PAR group additionally was given proprioceptive stimulation congruent with the observed movement. Ten trials were conducted for each movement type: 5 at spontaneous speed and 5 at slow speed, presented in a pseudorandom order to prevent anticipatory effects. At the end of each stimulation, participants were asked to replicate the movement as accurately as possible using the stimulated limb. Upon task completion, participants filled out a questionnaire assessing their sense of embodiment. Movement duration and joint angles were assessed and used to derive additional parameters, such as mean angular velocity (deg/s). Duration and mean angular velocity were compared with those of the observed videos, and absolute errors were calculated. Results showed that participants well imitated the observed movements for both VR and PAR protocols. Mean angular velocity error was lower in the PAR than in the VR protocol. However, these results were found to be statistically significant for elbow and not for wrist movements. Similarly, the sense of embodiment (Body Ownership, Sense of Agency and Location of the Body) was higher in the PAR than in the VR protocol only for elbow and not for wrist movements. In conclusion, the PAR protocol showed a significant effect in influencing temporal features of movement reproduction. Though, this effect was observed only in the body segment where the sense of embodiment was high.

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Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR075.09/M4

Topic: F.04. Voluntary Movements

Title: Glutamatergic Neurons in the Red Nucleus drive rapid movement arrest

Authors: *Y. KIM, H. H. YIN;
Dept. of Psychology and Neurosci., Duke Univ., Durham, NC

Abstract: The red nucleus (RN) is a conserved midbrain structure implicated in motor control, but its precise function in behavior remains poorly defined. Here we used optogenetics to bidirectionally manipulate vglut2-expressing RN neurons in mice performing a forelimb reaching task. Activation of RN glutamatergic neurons via ChR2 induced a rapid and sustained interruption of ongoing reaching behavior. In contrast, photoinhibition using GtACR produced a brief suppression at the onset of stimulation, with rapid recovery. In open field tests, ChR2 stimulation reduced locomotor activity, whereas GtACR had a transient suppressive effect. Retrograde tracing revealed that vgult2-expressing RN neurons receive excitatory input the cingulate cortex, prefrontal cortex, intralaminar and ventral thalamus, and superior colliculus. Anterograde tracing showed dense projections from the RN to the spinal cord, intralaminar thalamus, and subthalamic regions. Such connectivity partly resembles that of the basal ganglia, suggesting an alternative hub for the generation of volitional actions. Together, these findings suggest that RN glutamatergic neurons function as key node in motor control that is capable of halting voluntary movement, and that their precise temporal patterning may be critical for shaping adaptive motor output.

Disclosures: Y. Kim: None. **H.H. Yin:** None.

Poster

PSTR075: Reaching Control: Action and Sensation

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Topic: F.04. Voluntary Movements

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NIH Grant R01 NS111479

Title: A high-resolution robotic manipulandum for the study of sensorimotor learning in mice

Authors: *K. HUANG, T. BOLLU, M. PECK, Y. SUN, M. D. GOULDING, E. AZIM;
The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: The ability to learn and refine movements is essential for interacting with a rapidly changing environment and adjusting to changes that the body experiences. Motor adaptation - the

correction of learned, goal-directed movements to maintain performance when challenged with external or internal perturbations - is thought to involve multiple processes with distinct underlying mechanisms, each driven by different forms of errors. Furthermore, these processes can interact, with the outputs of one process influencing other processes to collectively effect changes in motor output. How these adaptation mechanisms eventually converge to influence movement at the neural circuit level remains an active and unresolved area of investigation. Addressing this challenge would be aided by the development of methods for differentially engaging and dissociating these distinct adaptation mechanisms at a behavioral level in genetically tractable mouse models. However, these efforts have been hampered by the difficulty of delivering the precise, fine-scale force perturbations that are required to manipulate the dynamics of the mouse forelimb. Inspired by devices commonly used in human and non-human primate studies as well as manipulandum tasks designed for rodents, we developed a high-resolution, closed-loop electronics system that controls a miniature robotic planar manipulandum for head-restrained mice. Our device can apply micro- and milli-Newton scale forces to the mouse forelimb with high temporal precision. We demonstrate the capabilities of this system by training mice to perform skilled goal-directed reaches on the manipulandum and assessing motor adaptation to different error-inducing perturbations, including velocity-dependent force fields and shifts in target location.

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Poster

PSTR075: Reaching Control: Action and Sensation

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Program #/Poster #: PSTR075.11/M6

Topic: F.04. Voluntary Movements

Support: NIGMS P20GM109098
NIGMS P30GM103503
NIGMS T32 AG052375
DOD No. W81XWH-21-1-0138

Title: Characterizing post-stroke muscle recruitment deficits and their modulation by functional electrical stimulation

Authors: *A. KOROL[1], A. ADCOCK[2], C. BRANDMEIR[3], P. ADELSON[4], V. GRITSENKO[3]; [1]Neurosci., [2]Neurol., [3]Human Performance - Physical Therapy, [4]Neurosurg., West Virginia Univ., Morgantown, WV

Abstract: Nearly 80% of stroke survivors experience upper limb motor impairments due to disruptions in neural circuits responsible for both gross reaching and fine motor tasks like grasping and manipulation. These tasks require two key control functions: tonic control to

counteract gravity and phasic control to manage intersegmental dynamics. The neural pathways involved include direct corticomotoneuronal projections and indirect corticofugal tracts that modulate brainstem motor outputs. After a stroke, reduced cortical drive often leads to compensatory overactivation of brainstem pathways, contributing to abnormal muscle synergies. This study aims to dissect how tonic (gravity-compensating) and phasic (dynamics-related) components of muscle activity reflect these control demands, how their recruitment patterns relate to motor deficit severity, and whether functional electrical stimulation (FES) of shoulder muscles can mitigate these impairments. In a series of cross-sectional studies, individuals with and without chronic post-stroke hemiparesis performed goal-directed reaching and grasping tasks toward virtual targets. Participants unable to reach completed shoulder flexion/extension movements instead. In select cases, FES was applied to the deltoid muscles to assist with antigravity support at the shoulder. Arm kinematics and surface EMG were recorded, and principal component analysis (PCA) was used to identify control strategies related to gravity compensation, intersegmental dynamics, hand opening, and spasticity. Results reveal distinct but interacting impairments in tonic and phasic muscle recruitment underlying individual motor deficits. Importantly, congruent shoulder FES altered distal muscle activity patterns and showed potential to reduce spasticity. These findings suggest that assessing and targeting specific components of motor control with neuromodulatory interventions like FES may offer a personalized approach to restoring functional movement in stroke survivors.

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Poster

PSTR075: Reaching Control: Action and Sensation

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Topic: F.04. Voluntary Movements

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WV NASA Space Consortium

Title: Dynamic modeling of neuromuscular interactions for motor performance across gravitational environments

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Abstract: Adaptation to altered gravitational environments, such as those experienced during spaceflight, significantly affects neuromuscular control and motor performance. To maintain functional movements, the nervous system must continuously adjust motor commands to generate muscle forces that counteract gravity and other external forces and manage inertial

interactions among limb segments. These forces—and the strategies used to produce them—vary substantially across individuals due to differences in musculoskeletal anatomy and neural sensorimotor control. Disentangling these neural and biomechanical sources of variability is essential for predicting individual performance and assessing health risks in space.In this study, we used musculoskeletal dynamic (MSD) modeling to quantify individual-specific motor strategies and predict neural control responses under varying gravitational conditions. In the first experiment, ten participants performed upper limb reaching tasks in upright and side-lying orientations while motion and surface electromyography (sEMG) data were recorded. Changes in muscle activity across orientations revealed gravity-dependent modulation not only in antigravity muscles but also in their antagonists. We then derived principal component scores to estimate the tonic (gravity-related) component of sEMG and used these to predict muscle activity under altered gravity conditions.In a second experiment, we applied this model to data collected during parabolic flights. Participants performed reaching movements while motion and sEMG data were collected under microgravity and hypergravity conditions. We constructed individualized MSD models of the arm and hand (27 DOFs) scaled to each participant's anthropometrics. Inverse simulations were used to compute muscle torques during different gravitational phases. Model-based predictions of muscle activity matched observed sEMG patterns across gravity conditions.These findings validate the utility of MSD modeling for predicting gravity-dependent changes in muscle recruitment and demonstrate its ability to reduce inter-subject variability by accounting for biomechanical differences. By capturing individual-specific neuromechanical profiles, this approach is particularly valuable in space science—where sample sizes are inherently limited—and offers a promising pathway toward personalized medicine applications on Earth.

Disclosures: M. McKendall: None. **O. White:** None. **V. Gritsenko:** None. **J. Babic:** None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.13/M8

Topic: F.04. Voluntary Movements

Support: U19 NS112959
F32 NS12623
RF1 NS128898
DP2 NS105555
R01 NS111479
Salk Pioneer Fund Postdoctoral Scholar Award
Salk Institute

Title: Flexible control of individual forelimb muscles underlies the control of movement speed

Authors: *A. NAGAMORI[1], T. BOLLU[2], A. R. THANAWALLA[3], M. D. GOULDING[4], E. AZIM[5];
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Abstract: The ability to regulate movement speed is critical for successful behaviors. Animals do so with apparent ease, despite the underlying computational complexity of neuromuscular control. The nervous system must control underdetermined, nonlinear musculoskeletal systems, which often offer no unique or exact solution that would allow animals to compute motor commands (i.e., muscle activation patterns) to produce desired movement kinematics. One prominent argument posits that the nervous system simplifies this control problem by recruiting and modulating fixed spatiotemporal patterns of muscle activity, thus reducing the dimensionality of control. We tested this proposed control strategy using a forelimb joystick reaching task in freely moving mice coupled with electromyography (EMG) recording , across 6-8 forelimb muscles. Using EMG and kinematic data, we built linear and nonlinear models to predict single-trial movement speed from muscle activity patterns and systematically tested hypothesized strategies of muscle coordination. We further tested these models against data in which chemogenetic activation/silencing of premotor, excitatory interneurons in the cervical spinal cord (V2a spinal interneurons) bidirectionally scales movement speed. We found that scaling of fixed spatiotemporal coordination does not fully account for trial-to-trial variation in movement speed. Instead, allowing flexible control of the amplitude and timing of individual muscles better explains trial-to-trial variation in movement speed and improves generalization to unseen data from V2a perturbation trials. Importantly, the relative importance of amplitude and timing control for adjusting movement speed varies across individual animals. These results suggest that the nervous system exploits, rather than reduces, the high dimensionality that flexible control of individual muscle coordination patterns offers. More generally, this work lays a foundation for defining how hierarchically organized sensorimotor circuits implement flexible control strategies to regulate movement speed.

Disclosures: A. Nagamori: None. **T. Bollu:** None. **A.R. Thanawalla:** None. **M.D. Goulding:** None. **E. Azim:** None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

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Topic: F.04. Voluntary Movements

Support: RF1 NS128898
U19 NS112959
DP2 NS105555
R01 NS111479
F31 NS130972

F32 NS126231
F32 NS120998

Title: Cerebellar pathways for rapid, bidirectional refinement of forelimb movement

Authors: *A. R. THANAWALLA[1], O. WILCOX[2], E. RHEE[1], J. JIANG[3], K. HUANG[1], R. YUSUFI[1], D. SAKLAWAY[1], A. NAGAMORI[1], A. I. CHEN[4], E. AZIM[1];
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Abstract: The cerebellum ensures the precise execution of dexterous forelimb movements, in part through the rapid online refinement of motor output. The cerebellar nuclei (CN) serve as the major output of the cerebellum, sending axonal projections that ascend and descend to diverse motor structures. The existence of these divergent pathways suggests that projections to distinct targets might have different roles in movement coordination. Yet, how discrete output pathways influence forelimb movement and contribute to online refinement remains unclear. Leveraging mouse molecular-genetic tools and intersectional viral approaches to target cerebellar nuclear neurons that project to the ventral anterior lateral complex of thalamus (CN^{VAL}), cervical spinal cord (CN^{cSC}), or both ($CN^{VAL/cSC}$), we found that CN projection neurons that target the VAL (CN^{VAL} and $CN^{VAL/cSC}$) are broadcasting populations that collateralize to several subcortical targets, forming distinct subnetworks within the larger population. Optogenetic perturbation coupled with kinematic and EMG analyses in a forelimb reach task uncovers distinct and antagonistic roles for these CN networks during forelimb movement, with discrete CN networks driving short-latency recruitment of muscle activity through disynaptic pathways to motor neurons to facilitate online refinement. Electrophysiological recordings during reaching reveal intermingled CN neurons whose activity correlates with and predicts either limb acceleration or deceleration throughout the movement. Together, this comparative approach reveals distinct CN networks that drive rapid bidirectional adjustment of forelimb movement, highlighting insights gained from pathway-specific functional dissection of cerebellar circuits.

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Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR075.15/M10

Topic: F.04. Voluntary Movements

Title: Dissecting the contribution of somatosensory corticofugal pathway to skilled reaching behavior

Authors: *X. XU;
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Abstract: Within the sensorimotor circuit underlying motor control, the primary somatosensory cortex (SSp) plays a critical role in processing afferent input and integrating sensory and motor signals necessary for skilled movement. While the classical view suggests that somatosensory information is relayed from SSp to motor cortex, which then issues motor commands via pyramidal tract (PT) neurons, the role of PT neurons within SSp itself during goal-directed behaviors remains less understood. This study examines the functional contributions of PT neurons in the upper-limb region of SSp (SSp-ul) during a naturalistic reach-grasp-to-drink task. Using a combination of knock-in mouse lines, two-photon calcium imaging, and closed-loop optogenetics, we characterize the activity patterns of SSp-ul PT neurons during active movement and passive somatosensory stimulation. Optogenetic inhibition is used to test the causal roles of SSp and its constituent cell types to skilled forelimb behavior performance. Our recent findings show that SSp-ul inhibition results in incomplete wrist supination and uncoordinated hand licking. This initial evidence underscores the critical role of SSp-ul in this behavior and motivates further investigation into the specific functions of its neural subpopulations. Preliminary data also suggest that distinct projection- and genetically defined populations in SSp-ul exhibit divergent activity patterns during forelimb movements, potentially reflecting differential roles in somatosensory processing and the control of precise motor output. This work offers new insight into how the cortical output pathways within a canonically-viewed somatosensory area contributes to motor control, which may aid in the development of neuroprosthetics, rehabilitation strategies, and treatments for motor dysfunctions.

Disclosures: X. Xu: None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.16/M11

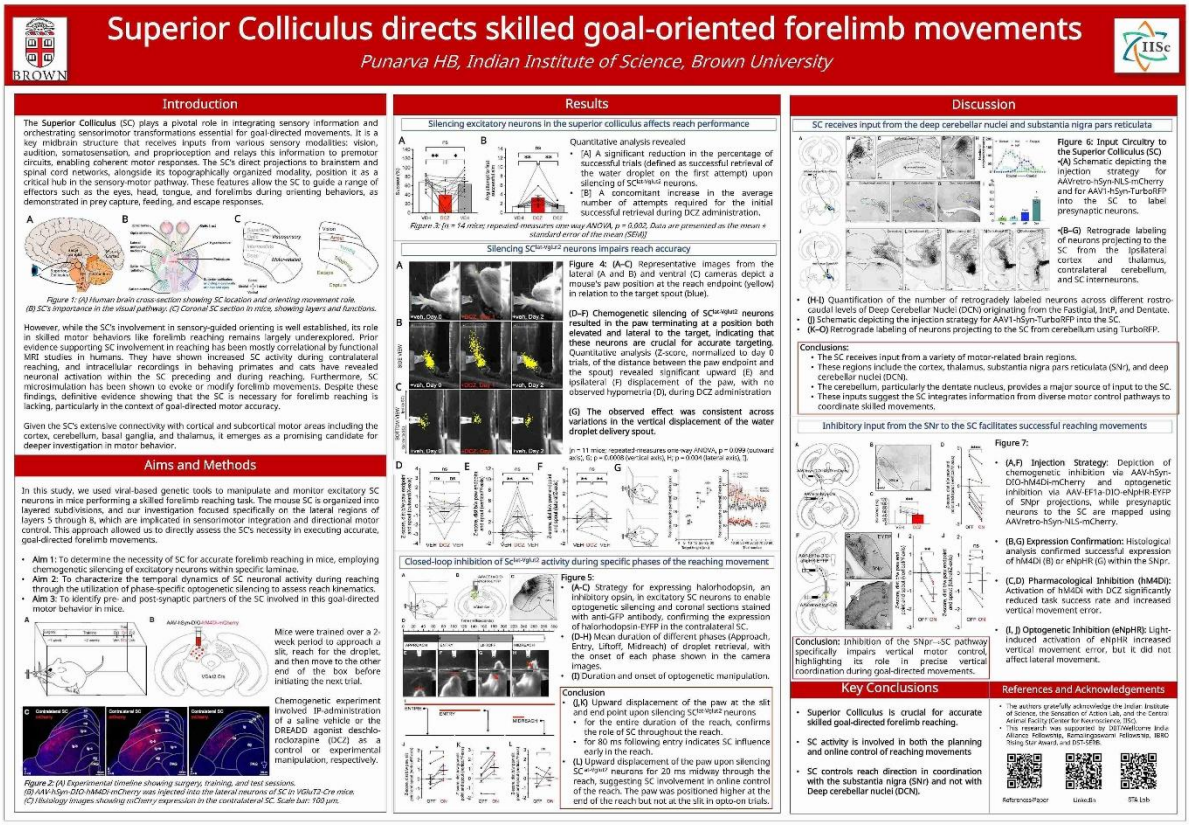
Topic: F.04. Voluntary Movements

Support: DBT/Wellcome Alliance
IBRO Rising Star Award
DST SERB

Title: Coordinated roles of superior colliculus and substantia nigra in skilled goal-oriented forelimb movements

Authors: *P. HARAPALLI BELLIAPPA[1,2];
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Abstract: Precise forelimb control is essential for goal-directed behaviors, yet the role of midbrain structures, particularly the superior colliculus (SC), in skilled movement remains underexplored. Traditionally linked to orienting responses, the SC in reaching is poorly understood despite containing key sensorimotor connections and preliminary fMRI evidence in humans showing activity during forelimb movement. Here, we identify a causal role for lateral SC excitatory (SClat-Vglut2+) neurons in controlling reach direction and endpoint precision during a skilled motor task. Vglut2-Cre mice were trained on an operant droplet retrieval task requiring accurate reach-to-grasp through a narrow slit. Chemogenetic silencing using AAV8-hSyn-DIO-hM4Di-mCherry and DCZ (0.1 mg/kg, i.p.) led to increased failure rates and reach attempts with upward and ipsilateral deviations. To examine SC activity, GCaMP8f was expressed in SClat-Vglut2 neurons and recorded via fiber photometry, revealing ramping prior to paw lift and peaking at reach termination, suggesting roles in both planning and execution. To test temporal causality, Halorhodopsin (eNpHR3.0) was expressed using AAV5-EF1α-DIO vectors, delivering closed-loop optogenetic inhibition via fiber-coupled lasers. Silencing SC during movement onset or mid-reach impaired trajectory accuracy. Kinematics were quantified with DeepLabCut for 2D paw tracking and endpoint precision. To identify modulators, retrograde AAVrg-Cre was injected into SC, and Cre-dependent fluorophores into the substantia nigra and deep cerebellar nuclei. Inhibition of the GABAergic SNr-SC (nigrotectal) projection induced downward, contralateral deviations, opposing those from SC silencing and indicating bidirectional tuning of movement vectors. Manipulating cerebellotectal inputs had no measurable effect. These findings reveal a midbrain locus for motor guidance and suggest potential deep brain stimulation targets for Parkinson's and motor control disorders.



Disclosures: P. Harapalli Belliappa: None.

Poster

PSTR075: Reaching Control: Action and Sensation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR075.17/M12

Topic: F.04. Voluntary Movements

Support: SIMONS
NSF GRFP

Title: Identification of a motor-related space in a premotor area in zebra finch

Authors: *I. DIPPENAAR[1], A. DUFFY[2], J. R. SCHERRER[3], J. WHITE[4], M. S. FEE[5], A. L. FAIRHALL[6];
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Abstract: Learned motor programs exhibit natural variability in their execution. While some motor variability may be due to noise, in male zebra finches the cortical-like nucleus LMAN injects variability into the song motor pathway, driving song variations across renditions. Previous work characterized the song-locked firing patterns of single units in LMAN, but it remains unknown how the population activity relates to song. Here, we report on the first population-level analysis of LMAN activity and quantify how neural variability in LMAN covaries with song fluctuations. We utilize canonical correlation analysis to identify directions of maximal covariance between LMAN activity and low-dimensional encodings of song. We identify a prevalent multidimensional motor-related subspace within LMAN that changes on a millisecond timescale over song and correlates with identifiable song features. The motor space significantly aligns with directions of high variability in both LMAN and song and often aligns most strongly with the direction of maximal variability. This study suggests that neural variability in LMAN is not isotropic, but instead structured to maximize its effect in driving variations in song.

Disclosures: I. Dippenaar: None. **A. Duffy:** None. **J.R. Scherrer:** None. **J. White:** None. **M.S. Fee:** None. **A.L. Fairhall:** None.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

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PRIN2022-2022BK2NPS

Title: From simulation to signal: a surgical planning workflow for chronic array recordings in the macaque premotor cortex

Authors: *M. DE VITIS[1], M. GAMBERINI[1], M. FILIPPINI[1], F. E. VACCARI[1], S. DIOMEDI[2], K. HADJIDIMITRAKIS[1], C. GALLETTI[1], P. FATTORI[1];
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Abstract: Surgical precision is crucial for chronic array implants in non-human primates (NHPs), particularly when targeting regions with limited cortical extent and/or located within sulcal banks. We developed and validated a comprehensive pre-surgical protocol integrating multimodal imaging (MRI/CT), 3D printing, CAD modeling, and real-time neuronavigation to simulate and optimize each step of cranial implant procedures in NHPs. MRI and CT scans provided subject-specific anatomical models, which were 3D-printed as skull replicas and combined with an agar-based brain phantom to perform dry-run simulations of the surgery, including implant testing and trajectory planning. Custom implant designs were modeled in 3D CAD, tailored to the cortical surface and imported into the planning environment. The full procedure was simulated using CORTEXPLORE SCI (cortEXplore GmbH, Austria), a markerless neuronavigation system combining infrared tracking and 3D anatomical rendering. Virtual implants and trajectories were overlaid onto 3D reconstructions of the anatomy, allowing iterative optimization before surgery. Intraoperatively, the same system enabled real-time tracking of instruments into MRI/CT volumes at submillimetric accuracy, ensuring accurate targeting. This pipeline was applied to implant a 120-channel Utah array (Blackrock Neurotech, Salt Lake City, UT) in the dorsal premotor cortex (PMd, area F2) of a female *Macaca fascicularis*, and to place a Precision Positioning System head holder (Thomas Recording GmbH, Germany). Neural recordings during a 3D instructed-delay reaching task confirmed broad tuning of F2 neurons to target direction and depth during movement preparation and execution, in line with previous F2 studies. This spatial profile was compared to that observed in medial posterior parietal cortex area V6A, anatomically connected with F2 (Gamberini et al., 2009), where responses are typically sharper and more selective (De Vitis et al., under review). Histology confirmed that the array was correctly placed in the F2 sector representing the upper limb, a critical target for neural recordings during reaching tasks. To further validate our protocol, we compared it to a previous implant placed without simulation guidance, where histology revealed only partial coverage of the target area. Our approach enabled anatomically specific sampling of F2 neurons, highlighting precision benefits from planning. This protocol improves implant accuracy and neural recording quality while supporting animal welfare and reproducibility, providing a generalizable framework for surgical planning in primate models.

Disclosures: M. De Vitis: None. **M. Gamberini:** None. **M. Filippini:** None. **F.E. Vaccari:** None. **S. Diomedi:** None. **K. Hadjidimitrakis:** None. **C. Galletti:** None. **P. Fattori:** None.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR076.02/M14

Topic: F.04. Voluntary Movements

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Nakajima Foundation fellowship
NIH R01 NS134634
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Title: A spatially distributed neural population facilitates motor computations through stable dynamics

Authors: *R. CANFIELD[1], T. OUCHI[2], B. MACAGNO[1], A. L. ORSBORN[2,4,3];
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Abstract: Performing a goal-directed movement is largely the result of two computations: planning and movement execution. On average, planning-related neural activity is first seen predominantly in pre-motor cortex (PM), with execution-related activity later appearing in primary motor cortex (M1). Yet, single-neuron activity patterns across these areas are spatiotemporally heterogeneous. Neurons that encode task information during planning or execution have been found in both PM and M1 and exhibit temporally complex activity patterns. Population level approaches have revealed structured preparation and execution computations but pool neurons from relatively small areas of frontal motor cortex, potentially missing structure in these computations related to neuronal spatiotemporal heterogeneity. It remains unclear how spatially distributed neural populations transition and transform between distinct, but linked, movement related computations. We examined how neural population computations are distributed across neurons in PM and M1 by making spatially precise recordings across the motor cortical surface using Neuropixels probes. We collected data from two monkeys while they performed a well learned delayed center-out reaching task. This allowed us to map functional contributions to preparation and execution across 3 spatial dimensions across a large area of frontal motor cortices; then assess single-unit and population level contributions to preparation and execution decoding and the dynamics between computations. We found that the neural populations with task information during preparation and execution have dynamics that show similar characteristics. When we compared these populations, we found neurons that

encode preparation clustered in PM and neurons that encode execution clustered in M1. However, we also found a spatially distributed subset of units with task information during both preparation and execution. We assessed the transformation between preparation and execution at the single-unit level and found expected tuning changes. However, at the population level we found that the unit group with task information during both preparation and execution had the most similar dynamics and similar task representation between computations. These results suggest that motor plans are transformed into actions through a transfer of information between spatially distinct and computation specific networks, linked by a spatially distributed subset of units. Furthermore, these results suggest that this spatially distributed subset of units facilitates the transition between distinct, but linked, computations through stable dynamics.

Disclosures: R. Canfield: None. **T. Ouchi:** None. **B. Macagno:** None. **A.L. Orsborn:** F. Consulting Fees (e.g., advisory boards); Meta Reality Labs.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR076.03/M15

Topic: F.04. Voluntary Movements

Title: Beta oscillations during motor preparation reflect neural dynamical state in macaque motor cortex

Authors: *Y. SUN[1,2], Y. ZHANG[2], H. CUI[1];
[1]Chinese Inst. for Brain Res., Beijing, China; [2]Inst. of Neuroscience, CAS, Shanghai City, China

Abstract: Although converging evidence suggests that beta oscillations (short for beta hereafter) in motor cortex decrease during movement execution, there is no a consensus on the role of beta in motor preparation. In the present study, we simultaneously recorded spikes and local field potentials (LFP) from macaque motor cortex via implanted Utah arrays (Monkey C, PMd, 128-ch, M1, 128-ch; Monkey P, PMd, 96-ch, M1, 96-ch; Monkey A, PMd, 64-ch, M1, 64-ch) while monkeys performed a flexible manual interception task, where monkeys should do once predictively center-out reaching for a circularly rotating target. Comparing beta activity in different target-speed conditions, we found significantly higher beta amplitude and higher probability occurred in static-target than in moving-target condition around GO cue (before movement onset) and we defined this time period as significant beta period. To elucidate the underlying mechanism, we analyzed simultaneously recorded population spiking activity through dimensionality reduction method (principal component analysis, PCA) and found that significant beta period marked the transition from movement preparation to execution in spiking neural trajectories. Besides, the speed of neural trajectory varied significantly with target speed and showed a negative correlation with beta probability at single-trial level. Notably, during significant beta period, the movement onset had a negative correlation with the speed of neural trajectory across all conditions, where reach for static target (higher beta) exhibited lower neural

speed and longer reaction time than reach for moving target (lower beta). Taken together, this work uncovers novel beta-related effects specific to reach toward moving target where the corresponding lower beta activity represents a more engaged neural state probably linked to the sensorimotor transformation for movement initiation whereas higher beta indicates a more idle neural state.

Disclosures: Y. Sun: None. **Y. Zhang:** None. **H. Cui:** None.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR076.04/M16

Topic: F.04. Voluntary Movements

Title: Motor Cortical Dynamics of Local Field Potentials and Spiking activity across Spatial Electrode Configurations during Reaching Arm Movements

Authors: *C. ZHENG, Y. XIAO, H. CUI;
Chinese Inst. for Brain Research, Beijing, Beijing, China

Abstract: As valuable markers for understanding network-level dynamics in cognition and disease, local field potentials (LFPs, low-frequency electrical signals recorded from the brain, typically 0.1-300 Hz) are increasingly being adopted as decoding signals in clinical applications. However, the dynamic differences between LFPs and spiking activity during reaching movements have yet to be fully clarified and defined. Here, we analyzed neural recordings from the motor cortex of rhesus monkeys (*Macaca mulatta*) using two electrode configurations: the linear 64-channel S-probe and the planar 96-channel Utah array. Monkeys performed delayed manual reaching tasks towards visual targets presented on a vertical touchscreen, either static (center-out) or moving along a circular trajectory (interception). Both single-unit activity (SUA) and entire spiking activity (ESA) exhibited sequence-like temporal patterns, as confirmed by jPCA analysis. Kalman filter-based decoding further demonstrated that these spiking features could linearly predict hand velocity with high accuracy. In contrast, local motor potential (LMP), a low-frequency component of the LFPs, did not show sequence-like activity but exhibited a high correlation with hand velocity. Interestingly, LMP patterns and decoding performance varied considerably across sessions and subjects, suggesting substantial heterogeneity. As expected, LFP power dynamics were frequency-dependent: both sequence-like patterns and decoding accuracy increased with frequency from 13-30 Hz to >200 Hz, although overall decoding performance remained lower than that of LMP or SUA. Moreover, recordings from the linear probes exhibited a layer-specific spectro-laminar organization, while neural features from planar Utah array showed more temporally dynamic and region-dependent distributions. These differences significantly affected LFP dynamics but had minimal influence on ESA, indicating that LFPs are more sensitive to the spatial configuration of recording electrodes. Taken together, our findings demonstrate that high-frequency (>120 Hz) LFP components more closely resemble

spiking activity in both sequence-like dynamics and linear correlation with kinematics. Conversely, these two features dissociate in LMP, which exhibits distinct dynamics that warrant further investigation. Our results also highlight the critical impact of electrode configuration on LFP dynamics, emphasizing the importance of careful electrode selection for both decoding applications and the interpretation of neural signals.

Disclosures: C. Zheng: None. **Y. Xiao:** None. **H. Cui:** None.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

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Program #/Poster #: PSTR076.05/N1

Topic: F.04. Voluntary Movements

Support: SFB 1538

Title: Intra-areal distribution of visuomotor modulation in the macaque fronto-parietal grasping network.

Authors: *R. NOCERINO[1], J. CHURAN[2], B. DANN[3], S. SCHAFFELHOFER[4], H. SCHERBERGER[3];
[1]German Primate Ctr., Göttingen, Germany; [2]Neurobio., [3]German Primate Ctr., Goettingen, Germany; [4]cortEXplore GmbH, Linz, Austria

Abstract: In everyday life, primates perform diverse grasping actions shaped by object properties such as fragility, texture, and weight. Accordingly, specific grip types are selected based on object features and intended actions. The frontoparietal grasping network—particularly the anterior intraparietal area (AIP) and ventral premotor cortex (F5)—is crucial for planning and executing such movements. While many studies have explored visuomotor transformations between these areas, intra-areal population dynamics remain poorly understood due to methodological limitations. To investigate intra-areal neural population differences, we trained a female *Macaca mulatta* to perform a visually instructed delayed grasping task using twelve objects presented on two PC-controlled turntables, eliciting diverse visuomotor transformations. Neural activity was recorded using Neuropixels probes (384 channels/day) at multiple AIP and F5 locations. Spike sorting was conducted with Kilosort4. We also applied the same analysis pipeline to a dataset recorded by Schaffelhofer et al. (2016), collected under identical task conditions using floating microelectrode arrays. In these datasets, we examined whether a visuomotor gradient exists across recording sites by computing the variance in spiking activity across conditions to assess the condition-dependent modulation strength in the neuronal population. In both AIP and F5, the movement epoch accounted for the greatest variance. Visual and preparatory epochs showed similar modulation levels, though preparatory activity was stronger in F5. In AIP, movement-related modulation decreased at more posterior/medial sites, while visual and preparatory modulation increased. In F5, a U-shaped gradient emerged during

the visual and preparatory epochs, with the strongest modulation at lateral and medial extremes. Movement-related modulation peaked laterally and declined medially. Data from the second monkey mirrored these trends. In AIP, visual and preparatory modulation increased at posterior/medial sites, while movement modulation remained high and constant. In F5, the U-shaped gradient persisted, and lateral sites again showed the strongest movement modulation. Despite anatomical differences, both datasets reveal that modulation strength varies nonlinearly across sites, highlighting localized tuning differences even within millimeters, and suggesting different functional subdomains in both the parietal and premotor grasping network.

Disclosures: R. Nocerino: None. **J. Churan:** None. **B. Dann:** None. **S. Schaffelhofer:** None. **H. Scherberger:** None.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

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Topic: F.04. Voluntary Movements

Support: NIH Grant R01NS129576

Title: Cortical sculpting of an ongoing motor rhythm

Authors: *E. KIRK, K. CAI, B. SAUERBREI;
Case Western Reserve Univ., Cleveland, OH

Abstract: The standard model of motor cortex maintains that muscle activity is a function of cortical firing rates. This model works well at explaining the neural control of voluntary movements such as reaching, but fails in cases where cortex must interact with a spinally-generated rhythm. During locomotion, the basic motor rhythm is produced by spinal networks, and the cortex acts to further sculpt muscle activity in response to environmental challenges. When an animal voluntarily modifies its gait to traverse a barrier, the motor cortex must transform information about obstacle proximity and the phase of the locomotor cycle into descending commands which alter kinematics in the correct limb at the right time.To determine how motor cortex implements this computation, we recorded activity in the forelimb area of mouse motor cortex using chronically implanted Neuropixels 2.0 probes. Animals were trained to trot on a linear treadmill and traverse obstacles attached to the belt. During unrestrained locomotion, three-dimensional motion tracking was used to segment step sequences relative to obstacles. Paw height was increased during gait modification as compared to locomotion over flat surfaces, and animals alternated between using the right and left forelimbs to initiate the traversal. Neuronal responses displayed rhythmic activity locked to stepping, transients near the obstacle, or complex mixtures of these patterns. We identified a preparatory neural dimension related to obstacle-proximity during the step sequence that becomes active regardless of which forelimb initiates the crossing. We found that the preparatory dimension is robust to optogenetic

silencing of the barrel cortex, whisker trimming and running in the dark, providing evidence that this dimension was not driven by a single sensory transient. Next, we identified a neural dimension related to the locomotor rhythm. We then asked whether this cortical rhythm generated by sensory feedback, or an efference copy from the central pattern generator. By linking the same neurons between locomotion and passive limb movements, we found that neural dimensions of the rhythm in cortex had minimal overlap between these conditions, suggesting the cortical rhythm is central, rather than sensory, in origin. We propose a new model whereby an input conveying obstacle proximity is gated by the locomotor rhythm to produce muscle-like execution dynamics that occur in the correct limb at the right time.

Disclosures: E. Kirk: None. **K. Cai:** None. **B. Sauerbrei:** None.

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

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Topic: F.04. Voluntary Movements

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UPenn Provost's Fellowship

Title: Neural population dynamics in posterior parietal cortex coordinate visuomotor behavior

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Abstract: Neuronal communication between brain regions underlies behavior in both health and disease, but how regional neuronal populations integrate and transform information remains an active area of investigation. Behaviors driven by temporally defined communication between brain regions may reveal fundamental properties that provide insight into brain activity during flexible behavior. Multiregional communication during visuomotor tasks can be measured behaviorally. When human subjects point toward a target, their ability to saccade away from the target diminishes (gaze anchoring), indicating inhibitory communication between reach and saccade networks. Neurophysiology experiments in non-human primates attributed gaze-anchoring to communication between the parietal reach region (PRR), an area involved in reach planning, and the lateral intraparietal region (LIP), an area involved in saccade targeting. In contrast to saccades cued without a prior reach, post-reach saccades were slowed due to PRR inhibition of LIP. To investigate how LIP neuronal populations integrate interregional input with local computations, we examined LIP neuronal population dynamics while animals were cued to saccade to a target either following a reach with variable delay or no reach. We hypothesized that neuronal subpopulations exhibiting reach and saccade related activity may communicate to coordinate gaze-anchoring. We found distinct subpopulations in LIP whose firing rates were primarily modulated by local saccade computations or additionally modulated by reach status.

Population activity during trials when a saccade was cued immediately following a reach occupied a non-overlapping subspace with population activity during trials without a prior reach. As the time between the reach and saccade increased, and expected PRR-LIP communication decreased, LIP population activity shifted from the former subspace towards the latter. To investigate the biological basis of subpopulation coordination, we estimated pairwise neuronal interactions in the context of the broader population dynamic with point-process generalized linear models. Clusters with significant fast (0.3-5 ms) interactions were more likely to respond to specific task events, indicating synaptic connections between subpopulations may facilitate task coordination. In summary, posterior parietal subpopulations driven by local specialization (saccade targets) and putative interregional input (reach history) exhibit dynamic interactions. Properties of coordinated neuronal population activity during gaze-anchoring may extend to behaviors where precise timing is harder to isolate.

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Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR076.08/N4

Topic: F.04. Voluntary Movements

Support: The Connected Minds Program

Title: Posterior intraparietal sulcus activity during a head unrestrained, memory guided reach task

Authors: *B. SHELDRICK;
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Abstract: The purpose of the current study is to investigate how local field potential (LFP) activity along the mid-posterior intraparietal sulcus (IPs) is modulated by visual landmarks before and during reaches to remembered visual targets. We recorded both action potentials and LFPs using 32-channel neural probes in one female Rhesus monkey. A landmark (four identical dots positioned at the vertices of a virtual square) was displayed at one of fifteen locations within reach on a touch screen. A visual target then appears, either within or outside of the landmark square, followed by a visual mask. After the mask disappeared, the landmark reappeared either at the same location (stable landmark condition) or shifted by 8 degrees in one of eight directions (landmark shift condition). Gaze and head position were allowed to move freely, and the animal was rewarded for reaching within 4.7 cm of the target. In the 'no-landmark' control trials, the procedure was the same, but the landmark is not presented. In parallel array recordings from posterior ventrolateral prefrontal cortex, LFP activity displayed decreases in delta band power during the memory-delay phase and an increase in both delta and theta power during the planning and execution of the reaching movement (Lin et al., *CAN-ACN* 2025). Preliminary

analysis of the current IPS LFP dataset suggests a decrease in beta band power that is time-locked to the reaching movement. Additionally, there appears to be an increase in delta and theta band power before reward in the landmark task conditions compared to no-landmark controls.

Disclosures: B. Sheldrick: None.

Poster

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR076.09/N5

Topic: F.04. Voluntary Movements

Title: Neural population dynamics of the macaque prefrontal cortex in predictive sensorimotor control

Authors: *Y. WEI[1], C. LI[1], Y. XIAO[1], H. CUI[2];
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Abstract: In daily life, sensorimotor control often relies on interaction with the dynamic environment, such as patting a flying mosquito or hitting a ball accurately. These processes engage the continuous integrating sensory information and translating it into flexible goal-directed behaviors. The prefrontal cortex (PFC) has long been thought to play a crucial role in integrating information and involved in broad aspects of executive behavioral control, which is upstream of sensorimotor transformation. To examine whether PFC neuronal activity proactively participates in predictive motor planning during dynamic tasks, we recorded population neuronal activity from the dorsolateral prefrontal cortex (dlPFC) and the primary motor cortex (M1) using Utah microelectrode arrays, while well-trained rhesus macaques performed flexible manual interceptions task. During the task, the monkey was instructed to perform reaching arm movements on a vertically oriented touchscreen to intercept circularly moving targets that appeared randomly at different positions and with four different velocities (clockwise/counterclockwise 120 deg/s, clockwise/counterclockwise 240 deg/s) or keeping static, and received a juice reward for success. We recorded an average of 50 single units per day from the dlPFC and 168 single units per day from the M1 across 4 sessions from one monkey. The tuning curves of the most dlPFC units were dramatically changed around the GO cue and at the movement onset (MO) period, indicating the dlPFC exhibited mixed coding and strong heterogeneity in different reaching direction and target motion velocity conditions. Moreover, Principal Component Analysis (PCA) showed that the neural trajectories in the dlPFC evolved from a clustered location in space to dispersed points from -800 (target onset, TO) to 600 ms (touch target, TT) around GO, unlike converging to a single location in a subspace as observed in M1, suggesting that the population dlPFC neural activity maintained representation to future reaching direction. Our results suggest that the PFC neurons indeed jointly tuned to sensory input and impending movement, indicating early abstract predictive motor planning to integrate

diverse information. Taken together, PFC neurons exhibited mixed coding of sensory and motor information, providing a persisted subspace representing the motor plan for upcoming movement.

Disclosures: Y. Wei: None. **C. Li:** None. **Y. Xiao:** None. **H. Cui:** None.

Poster

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Topic: F.04. Voluntary Movements

Support: NSF NCS DRL 2124066
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NIH R01-NS129098
NIH R01-NS129584

Title: Reward expectation encoding in prefrontal, premotor, and primary motor cortex is enhanced through learning

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Abstract: Reward expectations modulate behavior by affecting decision-making and movement execution. Consider a basketball player who needs to decide whether to pass or shoot. The player will need to estimate which decision is more likely to yield points (a reward). After making the decision, they must execute it with speed and precision. Both the decision-making process and the execution can be influenced by the player's ability to evaluate the consequences of the potential actions, which might develop with experience. We recently reported the coding of reward expectations in the motor cortex when monkeys perform reach movements to acquire rewards of variable sizes. Here, we aim to better understand the development of these signals with learning and relate them to decision-making and motor performance.
We trained a monkey to perform a challenging reaching task in which a letter symbol inside the target indicated the reward that would be received upon successful trial completion. In each session, we introduced new reward cues that had to be learned within the session, and for the sake of comparison, we also presented familiar reward cues with well-learned reward associations. For example, the letter S was consistently associated with a small reward in all sessions, but the letter H appeared only in a small subset of the sessions and was randomly assigned a reward value in each. We included trials with a single target, which allowed us to assess the motor performance to a specific reward cue. We also included choice trials in which the monkey selected between two targets with different reward values that allowed us to probe the monkey’s target preference and decision-making behavior. The monkey was able to learn the

novel reward cues within one session. Towards the end of the session, it reliably chose the larger reward cue in the two target trials, and showed shorter reaction times and higher success rates to larger reward cues.
While the monkey performed this task, we recorded from populations of neurons in the prefrontal cortex (area 8Ar), premotor cortex, and primary motor cortex. Early in the sessions, the neural activity in response to the presentation of the cues was separable by reward size between the old reward cues, but not between the new reward cues. Towards the end of the sessions, when the new cues were learned, they became separable as well. Our data provide insight into the way newly learned associations affect decision making, motor performance, and their neural correlates

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Poster

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Program #/Poster #: PSTR076.11/N7

Topic: F.04. Voluntary Movements

Support: The Warren Alpert Distinguished Scholar Award

Title: Motor cortical computation supporting free flight in bats

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Abstract: Understanding how the brain produces and controls complex, natural motor behaviors is one of the main challenges facing neuroscience. Although significant insights have been gained into motor cortical computations, much of this knowledge comes from tasks that require extensive training and constrain behavioral complexity. As such, they may not fully explore the computational space of natural dexterous movements. Bats are unique among mammals in having evolved true powered flight. Importantly, bat wings have evolved from extended flexible fingers providing control over more than 20 joint angles (hence the order name, Chiroptera or “hand-wing”). These hand structures confer precise dynamic adjustment of 3D wing shape and angle, suggesting sophisticated mechanisms of control. However, the motor cortical computations that support flight in bats remain unexplored. We combined an automated system for 3D pose estimation with Neuropixels recording of freely flying bats to explore the motor cortical computations that support flight. We find that Egyptian fruit bat flight consists of a series of fast (~8 Hz), diverse, and intricate wingbeat cycles, resulting in complex flight kinematics. However, bats naturally converge on a selection of highly reproducible flights paths. Flights within each repeated path are highly reproducible, with individual wingbeats in the same flight

phase exhibiting remarkably consistent kinematics. Thus, despite its complexity, bat flight is a strictly reproducible motor behavior facilitating analysis. Wireless Neuropixels recordings show most neurons do not display simple firing modulation along every wingbeat. Instead, we find adjustable wingbeat phase locking related to wingbeat kinematics. Indeed, many neurons were only recruited to specific wingbeat phases, often lasting under 50ms, at specific times during flight, or under specific kinematic conditions. At the scale of the whole flight, single units also showed highly diverse yet self-consistent modulation of firing rates, explained using encoding models to relate to continuous adaptations of specific joints across several wingbeats. This diversity resulted in a relatively high-dimensional population code that scaled with flight complexity. Together, these findings show bat motor cortex produces fast (~125ms), highly flexible and precise sequences at the level of individual wingbeats, where units are selectively recruited to specific wingbeat phases depending on exact kinematic needs. This flexibility may serve more generally as a basis for the flexible control of dexterous movements produced by motor cortex

Disclosures: B. Styr: None. **K.K. Qi:** None. **X. Chen:** None. **M.M. Yartsev:** None.

Poster

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Program #/Poster #: PSTR076.12/N8

Topic: F.04. Voluntary Movements

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Title: Encoding visuomotor transformations: Representation Similarity Analysis reveals task-dependent representational dynamics across macaque posterior parietal areas V6A and PEc

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Abstract: Within the posterior parietal cortex (PPC), areas in the medial PPC are engaged in eye-hand coordination during reaching tasks and exhibit a progressive transition from visuomotor to somato-motor reference frames along the posterior-anterior axis. We investigated whether the dynamics of space encoding for sensorimotor integration across different nodes of medial PPC are uniform or not.
To address this question, we recorded single-unit activity from areas V6A and PEc of the

macaque PPC while an animal performed three tasks designed to dissociate arm and gaze-related signals across directions and depths: in the foveated reach task, the monkey fixated and reached toward the same target; in the peripheral reach task, it maintained central fixation while reaching to peripheral targets; in the constant reach task, it fixated visual targets that were in different spatial locations, but reached to a central, constant location.
Representational Similarity Analysis was used to assess how spatial modulation related to gaze and arm position was encoded along the trial duration and within different areas. Significance of representational dissimilarity time courses was tested at each 50 ms bin ($p < 0.05$).
In V6A, early spatial modulation related to the fixation target emerged when the fixation point was cued; this information peaked around 800ms after this event and remained stable until the end of the trial. Instead, this information was available slightly later (approx. 50ms), with an earlier (approx. 50ms before than V6A), but weaker, peak in PEc. This suggests a long-lasting gaze-related modulation in V6A.
Subsequently, reach target signals encoded in a retinotopic reference frame (i.e. relative to the gaze position) appeared as soon as the monkey was instructed about the reaching goal in V6A, but later (by about 500 ms) in PEc. In V6A, this touch target signal encoded in gaze coordinates increased steadily during the delay phase, peaking around 500 ms before movement onset indicating a strong preparatory activity, and then gradually declined. This progression reflects a dynamic, gradual shift from purely gaze-related tuning to the encoding of motor goals in retinotopic coordinates. PEc displayed a delayed onset timing, and also a much delayed peak during the hold phase. Overall, both areas showed early gaze-related responses followed by reach target encoding in gaze coordinates. V6A, with stronger oculomotor signals than PEc, encoded spatial information more stably. This supports a functional gradient in spatial visuomotor transformations.

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Poster

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Program #/Poster #: PSTR076.13/N9

Topic: F.04. Voluntary Movements

Title: Executive regions encode movement through multiplexing and visual subspace modulation

Authors: *X. NIU[1,2], M. SLAPIK[3], A. G. MCCONNELL[1], V. DRAGOI[4];
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Abstract: The brain organizes functionally into distinct regions for vision, hearing, motor action and decision-making. This modularity raises important questions about how different regions interact to support complex behavior. To investigate this, we studied freely moving macaques performing foraging behaviors in an open-field enclosure. We performed wireless simultaneous recording of neural activity from dorsolateral (area dlPFC) and visual cortex (area V4) using Utah arrays, while tracking full-body movement using an overhead camera and using machine learning algorithms (DeepLabCut) to define behavioral and motor events. We found that V4 neurons contributed little to motor encoding whereas dlPFC neurons that communicated strongly with V4 subspaces achieved higher prediction accuracy. This suggests a modulatory influence of visual inputs used for motor encoding. Additionally, dlPFC neurons can multiplex posture and location information such that distinct neural clusters support memory and planning functions. Task engagement and reward-based learning further improved decoding accuracy, highlighting the role of dlPFC in credit assignment and goal-directed control. This work reveals how visual and executive regions interact during natural behavior, and how the primate prefrontal cortex integrates motor, cognitive, and sensory signals to guide flexible sensory-motor action.

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Poster

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Program #/Poster #: PSTR076.14/N10

Topic: F.04. Voluntary Movements

Title: Task-performance interactions in Ventrolateral Prefrontal Cortex LFP activity during a head-unrestrained, memory guided reach task

Authors: *J. LIN[1], V. NÁCHER[2], H. WANG[3], X. YAN[4], J. C. MARTINEZ-TRUJILLO[6], J. CRAWFORD[5];
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Abstract: The purpose of the current study is to understand how prefrontal local field potential (LFP) signals encode visual landmark information during a memory-guided reach task, and how this is modulated by performance (i.e., correct vs. incorrect trials). We implanted a 128-channel Plexon Array over the posterior ventrolateral prefrontal cortex (pVLPFC). Single-unit activity, LFPs and behavioral (eye, head, hand) signals were recorded simultaneously in a female Rhesus monkey trained to perform a memory guided reaching task. In this task, the hand was initially placed at a waist-level LED bar while gaze fixated centrally, and a landmark (four dots forming a virtual square) was presented at one of 15 locations on a touchscreen. A visual target then appeared transiently at a variable location within or outside this virtual square, followed by a

visual mask. After the mask, the landmark either reappeared at the same location (stable landmark condition) or shifted by 8 degrees in one of 8 directions (landmark shift condition). The fixation light then extinguished, signaling a reach to the target. Controls were the same, but without the landmark. Preliminary analysis of time-power plots revealed frequency band-specific decreases in power during the delay period before reach, followed by increases in power peaking near movement onset across all trials. Correct stable landmark trials (which tend to be more accurate than no-landmark controls) and landmark shift trials showed additional band-specific decreases and increases in power prior to movement onset, plus a second power peak between acquisition and reward. However, the latter landmark-specific changes appeared to be suppressed during error trials where the animal touched the wrong location. These preliminary findings suggest that prefrontal LFP signals contain visual context-dependent information for reach planning and execution, and that the absence of this visual information may be associated with errors in performance.

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Poster

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Topic: F.04. Voluntary Movements

Support: NINDS Grant UH3NS109845
Medtronic RC+S Summit system donated by Medtronic

Title: Thalamic biomarkers for adaptive deep brain stimulation in Essential Tremor

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Abstract: Adaptive deep brain stimulation (DBS) is a promising approach for alleviating the refractory, debilitating symptoms of Essential Tremor (ET). We investigated whether local field potentials (LFP) recorded from the thalamic ventral-oralis (VO) nuclei could control DBS in the ventral intermediate (VIM) nucleus. Three ET patients were implanted contralaterally with dual Medtronic Summit RC+S leads in VIM and VO. VO LFPs were sampled while patients performed Rest, Posture, and Spiral drawing. VIM DBS was alternately On and Off while contralateral arm acceleration was concurrently recorded.
LFP band powers in the Delta, Theta, Alpha, Beta, Low Beta, Mid Beta, and High Beta ranges were extracted. Within each DBS state, permutation tests and binomial generalized linear mixed-

effects models (GLMMs) were used to classify Rest v. each behavior, reporting AUC with bootstrapped confidence intervals. To predict tremor severity, accelerometry power in the task 2 Hz tremor band was correlated with LFP percent power in each band and with that LFP tremor band (TB) via Gamma log GLMMs. Fisher Z gave 95% CIs on Pearson coefficients.
Under both DBS Off and DBS On, Delta power increased with movement ($p \leq 0.0485$), whereas Alpha and all sub-Beta bands decreased (Off: Alpha $p = 0.007$ v. Spiral; Beta $p < 0.001$ v. both behaviors; On: Alpha $p < 0.001$ v. Spiral; Beta $p < 0.001$ v. both). Comparing Off to On, Rest showed no band power changes ($p > 0.3$), but Spiral and Posture exhibited additional Beta suppression (Spiral $p = 0.006$; Posture $p < 0.001$) and Spiral alone showed further Alpha decrease ($p = 0.002$). In classifying Rest v. Spiral, Delta and Alpha were strongest (Off: AUC 0.713/0.707; On: AUC = 0.691/ 0.709). Low Beta and Beta performed moderately (Off AUC ≈ 0.68/0.64; On ≈ 0.63/0.56) while High-Beta became significant only On (AUC = 0.611). In Rest v. Posture, Off Delta and Beta (AUC = 0.637/0.672) led, with Low Beta and Mid Beta also significant. Under DBS On, Delta and Beta fell to chance levels (AUC < 0.58).
Tremor severity correlated strongly with all bands Off DBS (TB $r = 0.469$; Beta $r = 0.444$; Low Beta $r = 0.423$; all $p < 10^{-98}$). Under DBS On, TB ($r = 0.182$), Low Beta ($r = 0.169$), Delta ($r = 0.100$), and Alpha ($r = 0.094$) remained significant ($p < 10^{-5}$), while Theta was marginal ($r = 0.043$, $p = 0.00004$). All others were not significant.

Significance

VO LFP reliably tracks both movement and tremor. Under DBS Off, Beta sub bands and an individualized Tremor Band strongly distinguished motion and predicted tremor. DBS On attenuated Beta's tremor prediction but not Delta and Alpha's. A personalized Tremor Band and Low Beta continued to track tremor under DBS On. These findings support the potential of VO LFP to drive VIM DBS in ET.

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Poster

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Program #/Poster #: PSTR076.16/N12

Topic: F.04. Voluntary Movements

Support: NIH grant support #NS93909

Title: Neurons in mouse posterior parietal cortex adapt temporal representation of choice, but not sensory cues, to fit anticipated reward timing

Authors: *P. N. ATTYA[1], B. GOOLSBY[2], B. A. ABDULLAH[1], S. ELDAWLATLY[3,1], K. G. OWEISS[4];
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Abstract: Cognitive flexibility allows the brain to maintain information related to reward seeking behavior while continuously adapting to emerging task demands. Reward prediction models posit that time to anticipated reward is key to this adaptation. Yet, it is not entirely clear how the mnemonic encoding of retrospective sensory information or the prospective encoding of planned actions in delayed choice tasks are affected by time to reward. Here we trained head-fixed adult mice (n = 7) on an auditory 'cloud of tones' categorization task in which animals had to lick left or right for low (1-5 KHz) or high (10-20 KHz) frequency tones (10 tones, 37.5 ms each), respectively. We used two photon Ca^{2+} imaging to characterize the responses of over 3000 neurons in the Posterior Parietal Cortex (PPC) under two contexts: immediate (C1) or delayed reward (C2). We then trained Long Short-Term Memory (LSTM) networks (50 models per session/context) to decode either cue category or choice from population activity dynamics. LSTMs trained on C1 trials and tested on C2 trials of the same session achieved high cross-context accuracy (>90%) during the 375 ms cue epoch in C2, but gradually dropped (<80%) as time progressed through the delay interval, suggesting that sensory encoding remained similar during early delay epoch but became dissimilar towards motor planning epochs. We used demixed Principal Component Analysis (dPCA) to separate variance along stimulus, decision, and reward axis (C1 vs C2) as well as condition-independent components. We found that the proportion of variance along each axis largely varied across different populations and sessions. Higher cue or choice related components exhibited stronger cross-context LSTM decoding, whereas sessions dominated by reward or condition-independent variance showed poorer decodability (<60%). These results suggest that sensory, choice and time to reward representations are intermixed in PPC, and that mnemonic encoding of sensory cues remains stable. Choice encoding and motor planning, however, encode the time to reward by slowing down evolving neural population trajectories, eventually reaching choice predictive states around the time when reward becomes available. Our results demonstrate that neurons in PPC can flexibly switch their computational roles between different cognitive demands of the same task.

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Poster

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Program #/Poster #: PSTR076.17/N13

Topic: F.04. Voluntary Movements

Title: All-optical interrogation of cellular and population mechanisms subserving implantable brain-computer interface learning and skill consolidation

Authors: *S.-Y. CHUEH[1], K. G. OWEISS[2];
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Abstract: Learning brain-computer interface (BCI) control relies on rapid but intricate mechanisms of intrinsic and synaptic plasticity taking place at the cellular and population levels. Quantifying these mechanisms in vivo, however, remains a real challenge. Here, we developed an all-optical approach to probe these mechanisms at various timescales spanning seconds to minutes to hours and days. Transgenic mice expressing GCaMP7s and soma-targeted opsin ChRmine-Kv2.1 in excitatory neurons of visual cortex (V1) layer 2/3 were longitudinally imaged across multiple BCI training sessions. Using cell targeted optogenetic stimulation, we first artificially induced intrinsic plasticity (IP) to characterize how individual neurons alter their spiking frequency-input (f-I) functions upon exposure to constant depolarizing inputs that maintained their spiking outputs at chosen target levels akin to BCI modulation. We then validated the observed shifts in these functions in a subset of experiments using two-photon-guided whole-cell recordings. Artificial IP induction (n=7 cells, N=3 mice) showed significant changes in amplitude and time constant of evoked somatic Ca^{2+} transients ($p < 0.05$) that lasted for a few minutes, consistent with models of spike-frequency adaptation. Notably, return to homeostatic state following induction was target-level dependent, suggesting rapid and transient memory formation of depolarizing input history.We then characterized how intrinsic plasticity could play a role in BCI learning and skill consolidation when embedded within a BCI ensemble. We trained different BCI ensembles in the same animal to control a one-dimensional cursor for reward and analyzed the concurrent changes in intrinsic excitability and synaptic plasticity within BCI neurons as well as non-BCI neurons as learning progressed. Correlation among BCI neurons showed significant decline across sessions ($p = 0.032$), suggesting that BCI control may consolidate the control around IP rather than synchronous modulation when limited coordination is available within the ensemble. Conversely, it may rely less on IP when local coordination between synaptically coupled neurons is available. Furthermore, ensembles achieving rapid performance gains predicted stable BCI performance in later sessions. Taken together, our results support a meta plasticity model linking cellular and ensemble adaptation to learning in cortical networks.

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Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR076.18/N14

Topic: F.04. Voluntary Movements

Support: NINDS Grant UF3NS109845
Medtronic RC+S Summit system donated by Medtronic

Title: Long-term outcomes of unilateral thalamic deep brain stimulation in essential tremor: disentangling disease progression from dbs tolerance

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Abstract: Background: Essential Tremor (ET) is historically the most prevalent movement disorder in adults. Ventral Intermediate Nucleus Deep Brain Stimulation (VIM-DBS) provides substantial symptom relief; however, about 50% of patients experience diminishing benefits over time. The underlying causes, ranging from disease progression to DBS habituation and tolerance, remain poorly understood. **Methods:** We performed a retrospective longitudinal analysis of 87 ET patients (mean age: 68.7) with unilateral VIM DBS and at least 2 years of follow-up (mean: 6.3 years; max: 15.4 years). Tremor severity was quantified using the Fahn-Tolosa-Marin Tremor Rating Scale (TRS), including contralateral (CUE) and ipsilateral (IUE) upper extremities subscores. Symptom trajectories were analyzed by DBS state (ON vs. OFF) and time phase. **Results:** In the early phase (<21 months), 90% of patients showed substantial improvement. Beyond this period, 66% experienced symptom worsening. Analysis of CUE and IUE TRS scores in the DBS-OFF condition revealed comparable worsening in both contralateral and ipsilateral sides, suggesting no lateral asymmetry in disease progression. Moreover, IUE TRS trajectories during DBS-ON and OFF states were similar, suggesting the non-stimulated side is not significantly affected by CUE DBS, thereby validating its use as a stable internal reference for normalization. Normalized CUE/IUE scores in the DBS-ON state showed no significant long-term decline, implying that the apparent deterioration in raw scores is primarily attributable to disease progression rather than a loss of DBS efficacy. Similar trends observed in both DBS-ON and OFF trajectories further support this interpretation indicating sustained DBS effects. **Conclusions:** Long-term VIM-DBS outcomes in ET suggest that symptom deterioration over time is more consistent with underlying disease progression than with DBS tolerance. Using the non-stimulated side score as a normalization reference enables patients to serve as their own internal control. Importantly, regular clinical reassessment and stimulation parameter optimization may mitigate potential DBS habituation effects. These findings underscore the importance of extended comprehensive outcome assessment in ET, incorporating not only TRS scores but also more objective tremor quantification to enhance the evaluation of long-term therapeutic efficacy.

Disclosures: N. Bakri: None. **K.G. Oweiss:** 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 Grant UH3NS109845. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic (Minneapolis, MN).

Poster

PSTR076: Cortical Planning and Execution: Neurophysiology

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR076.19/N15

Topic: F.04. Voluntary Movements

Title: Tdcs improves performance on near transfer tasks in left-handed individuals

Authors: A. HELMS[1], T. HERRIN[2], J. BEANE[1], C. JONES[3], J. SENTMAN[3], *L. LIMA DE ALBUQUERQUE[4];
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Abstract: Transcranial direct current stimulation (tDCS) has shown promise in enhancing motor skills and learning for relatively simple motor tasks that involve the hand and arm. Nevertheless, a limited number of studies have explored the impact of tDCS on the motor performance related to complex, multi-joint tasks that require coordination across the entire body. Available studies excluded left-handed individuals because of significant variability in their neurophysiology and brain anatomy. Therefore, the purpose of this study was to examine the influence of tDCS on near transfer tasks after a 20-minute practice of an overhand throwing task with the non-dominant hand performed by left-handed individuals. The study was a randomized, SHAM-controlled, within-participants, double-blind experimental design. A total of 12 left-handed participants completed a tDCS condition and a SHAM condition in two experimental sessions separated by a 7-day washout period in a randomized manner. In each session, participants were assessed using the Purdue Pegboard and Jebsen Taylor Hand Function Tests (JTT) before and after practice. Each subtest was performed with the right non-dominant hand, followed by the left dominant hand. A retention test block was administered 24 hours later in the same fashion. Anodal tDCS was applied to the left motor cortex over the first dorsal interosseus (FDI) muscle representational area during the overhand task practice blocks for 20 minutes. tDCS was delivered using previously determined effective parameters (current strength: 1 mA; anode: FDI; cathode: contralateral supraorbital area) with a NeuroConn stimulator. Pegboard performance was measured by the total number of pegs placed in 30 seconds. The total time to complete all subtests in JTT (excluding handwriting) was quantified as a variable for each hand. A three-way (Tests, Hands, Stimulation) repeated measures ANOVA was used for statistical analyses. All interactions were significant for the pegboard (all P values < 0.05), except for *test*stimulation* ($F(2,20) = 2.745$, $P = 0.088$, $\eta 2 = 0.215$), indicating better performance with both hands for both groups. Similarly, all interactions were significant for JTT, except for *hand*stimulation* ($F(1,11) = 1.169$, $P = 0.303$, $\eta 2 = 0.096$), indicating that participants were significantly faster on JTT with both non-dominant and dominant hands. Since the *test*hand*stimulation* interactions were significant for both assessments, these preliminary findings indicate that a single application of tDCS has the capacity to improve performance on near transfer tests in left-handed individuals.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: PSTR077.01/N16

Topic: F.04. Voluntary Movements

Title: Motor and non-motor features of performance fatigability in parkinson's disease and healthy controls.

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Abstract: Performance fatigability refers to the objective, measurable decline in performance over time during a sustained or repeated task. Exacerbated performance fatigability is often observed in movement disorders such as Parkinson's disease (PD). This ongoing study aims to examine how motor and non-motor features contribute to performance fatigability in individuals with PD compared with healthy controls (HC). Understanding the multidimensional nature of performance fatigability is critical to investigate underlying neural as well as psychological mechanisms of performance fatiguability. Here, we examined performance fatigability using a bilateral upper limb time-to-failure task. We analyzed data from 4 individuals with Parkinson's disease (age: 68.6 ± 7.43 years) and 4 healthy controls (age: 64.8 ± 9.55 years). Participants sustained 90° shoulder flexion while projecting lasers within a 3 cm target radius on a screen 2 meters away. This task was repeated five times. For each trial, we recorded time to task failure (TTF), laser trajectory, and surface EMG from the upper limb and trunk muscles. We also assessed perceived exertion, mood-related disorders, cognitive status, as well as motor performance measures such as peak force error during a fast goal directed task, reaction time variability during a GO/NOGO task, and maximum voluntary contraction (MVC) for shoulder flexion and ankle dorsiflexion. Our findings indicate that on average, PD showed significantly shorter TTF (93.22 s) compared with HC (183.16 s). In early trials, TTF was primarily associated with non-motor features, including apathy (R2=0.25), depression (R2=0.37), and anxiety (R2=0.67). In contrast, TTF in later trials was strongly related to motor features such as peak force error (R2=0.70) and changes in shoulder maximal voluntary contraction (R2=0.32).The exacerbated fatigability in PD relative to HC in early trials is primarily associated with non-motor features, whereas at late trials exacerbated fatigability is primarily associated with motor features.

Disclosures: R.J. Malik: None. **J.J. Kim:** None. **A. Chagas Pereira:** None. **V.R. Shankar:** None. **E.A. Christou:** None. **B. Yacoubi Keyhani:** None.

Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR077.02/O1

Topic: F.04. Voluntary Movements

Support: NIH R01-NS058487

Title: Bradykinesia and muscle activity in Parkinson's disease

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Abstract: A well-known symptom of Parkinson's disease (PD) is slowness of movement (bradykinesia). Few studies have analyzed the muscle activity of individuals with PD performing voluntary open-loop contractions. The purpose of this study was to compare the time-to-peak force and EMG activity of the primary agonist muscle during fast goal-directed force contractions (15% of maximum force in 160 ms) between PD and healthy controls (HC). Participants performed an index finger abduction task (PD n=39, HC n=35) and an ankle dorsiflexion task (PD n=36, HC n=33). From the force output, we quantified peak force (PF), peak force error (PFE), time-to-peak force (TPF), and time-to-peak force error (TPFE). From the EMG activity of the first dorsal interosseus (FDI) and tibialis anterior (TA), we quantified peak EMG burst (EMG_{Peak}), time to peak EMG burst (EMG_{TP}), and modulation of the EMG burst (EMG_{MOD}) from 12-35 and 35-60 Hz using wavelet analysis. We found that individuals with PD exhibit longer TPF ($p<0.001$) for both contractions. Individuals with PD also exhibited longer EMG_{TP} in the TA contraction ($p<0.001$) and greater EMG_{MOD} from 12-35 Hz ($p=0.001$) for both contractions. Greater EMG_{MOD} from 12-35 Hz positively associated with longer TPF (Ankle $R^2=0.227$, Finger $R^2=0.3049$). These findings show that greater modulation of the agonist EMG burst from 12-35 Hz is related to bradykinetic behavior in individuals with PD during fast goal-directed force contractions. Similarity in findings for the index finger and ankle highlights the central origin of the altered muscle activity in PD.

Disclosures: T.W. Worthington: None. **J.J. Kim:** None. **S. Delmas:** None. **R.J. Malik:** None. **V.R. Shankar:** None. **A.M.A. Chagas:** None. **W. Sharma:** None. **J. Stone:** None. **E.R. Tobin:** None. **D.E. Vaillancourt:** A. Employment/Salary (full or part-time): University of Florida. **B. Yacoubi Keyhani:** A. Employment/Salary (full or part-time): University of Florida. **E.A. Christou:** A. Employment/Salary (full or part-time): University of Florida.

Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Topic: F.04. Voluntary Movements

Support: NIH R01-NS058487

Title: Force tremor as an early marker for Parkinson's Disease

Authors: *A. CHAGAS[1], J. J. KIM[1], R. J. MALIK[1], V. R. SHANKAR[1], T. WORTHINGTON[1], E. R. TOBIN[1], B. YACOUBI KEYHANI[2], D. E. VAILLANCOURT[1], E. A. CHRISTOU[3]; [1]Applied Physiol. and Kinesiology, [2]Applied Phsio and Kinesiology, [3]Applied Physiol. & Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: Parkinson's Disease (PD) is a prevalent neurodegenerative disorder that causes motor impairments such as bradykinesia, rigidity, and tremor. The diagnosis of PD is based on the motor and non-motor symptoms the person experiences over time. Evidence shows that a person starts perceiving the symptoms, when ~50% of the cells in the substantia nigra are already degenerated, which corresponds to years of degeneration. An earlier PD diagnosis—before neurodegeneration begins—can enable neuroprotective treatment strategies, potentially slowing the progression of motor symptoms. Rapid Eye Movement Behavior Sleep Disorder (RBD), a parasomnia marked by dream-enacting behaviors, is classified as a prodromal condition for PD, as more than half of those with RBD eventually develop PD. Therefore, RBD patients offer a unique opportunity to identify early motor dysfunction that precedes a PD diagnosis. In this study, we aim to determine whether hand or ankle force tremor in individuals with RBD is significantly exacerbated compared with healthy controls (HC). We investigated 34 healthy HC (63.35 ± 7.40 years, 18 males), 40 RBD (60.89 ± 8.79 years, 27 males), and 39 early-stage PD (62.58 ± 7.78 years, 22 males). Each participant performed the same force task executing index finger abduction and ankle dorsiflexion. The task required maintaining a constant force at 15% of their maximum voluntary contraction for 120 seconds across three trials. Tremor was quantified as the ratio of force output power within the 4-8 Hz band to the total force power within the 1-12 Hz range. For the index finger abduction, 34 HC, 39 RBD, and 38 PD were analyzed. The force tremor in RBD was not significantly different from HC ($p = 0.41$; 19.1 ± 1.43 % vs. 18.6 ± 1.64 %). PD exhibited significantly greater tremor (26.8 ± 2.15 %) than RBD ($p = 0.004$) and HC ($p = 0.003$). For the ankle dorsiflexion task, we analyzed 33 HC, 38 RBD, and 36 PD. RBD exhibited significantly greater tremor than HC ($p = 0.04$; 9.12 ± 0.81 % vs. 7.24 ± 0.70 %), and significantly lower tremor than PD ($p = 0.003$; 9.12 ± 0.81 % vs. 16.58 ± 2.26 %). Additionally, HC exhibited significantly lower tremor than PD ($p = 0.0003$). These novel findings indicate that ankle tremor may serve as one of the earliest detectable markers of motor dysfunction in RBD, potentially signaling the onset of PD before clinical diagnosis.

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Poster

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Topic: F.04. Voluntary Movements

Support: NIH R01-NS058487 to DEV

Title: Vocal Deficits in Rapid Eye Movement Sleep Behavior Disorder: A Potential Early Marker for Parkinson's Disease

Authors: *V. R. SHANKAR, J. J. KIM, R. J. MALIK, A. M. CHAGAS, T. W. WORTHINGTON, S. DELMAS, E. R. TOBIN, D. E. VAILLANCOURT, B. YACOUBI KEYHANI, E. A. CHRISTOU;
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Abstract: Parkinson's Disease (PD) diagnosis has traditionally relied on motor symptoms such as bradykinesia, rigidity, and tremor, which become evident only after significant neurodegeneration. Early detection of PD is urgently needed to develop treatments that prevent disease progression prior to diagnosis. Rapid Eye Movement Sleep Behavior Disorder (RBD), a known precursor to PD, provides a unique population to investigate early predictive markers. Previous research demonstrates that PD patients exhibit hypokinetic dysarthria primarily evident as hypophonia (reduced loudness) and monopitch (reduced variation in fundamental frequency - F0; termed dysprosody). There is evidence of subtle voice impairments in RBD suggesting dysprosody. However, many F0 characteristics remain unexplored in RBD, including F0 tremor and inconsistency. We quantified several F0 characteristics during the sustained vowel /ū/ for 10 trials in 32 healthy controls (HC; 62.8 ± 7.6 Years, 14 F), 38 RBD (60.7 ± 8.5 Years, 8 F), and 36 PD patients (62.6 ± 8 Years,13 F). Participants sustained /ū/ using their normal intensity and increased intensity (twice the normal level). F0 characteristics included F0 frequency, F0 frequency range, and vocal tremor (modulation of F0 - power from 4-8 Hz relative to 1-12 Hz). In addition, trial-to-trial variability was quantified using the coefficient of variation (CV) of F0 frequency, F0 frequency range, and vocal tremor across the 10 trials. The F0 frequency and F0 frequency range were significantly different among the three groups only during the higher vocal intensity condition. The HC group exhibited significantly greater (all $p<0.05$) F0 frequency (HC: 242 ± 92 Hz, RBD: 168 ± 40 Hz, PD: 195 ± 57 Hz) and F0 frequency range (HC: 26 ± 17 Hz, RBD: 14 ± 9 Hz, PD:18 ± 9 Hz) than the RBD and PD groups. Vocal tremor and trial-to-trial variability outcomes were not significantly different (all $p>0.2$) across the three groups and vocal intensity levels. This study introduces novel findings showing that increasing vocal intensity during sustained phonation unmasks F0 deficits in RBD. These results suggest that assessing F0 frequency and its characteristics under high-intensity conditions may serve as an early marker for PD.

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Poster

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Topic: F.04. Voluntary Movements

Support: NIH R01-NS058487

Title: Impaired Retention of Goal-Directed Movements in Parkinson's Disease: A 2-Year Longitudinal Analysis

Authors: *J. J. KIM[1], S. DELMAS[1], E. R. TOBIN[1], R. J. MALIK[1], V. R. SHANKAR[1], A. M. CHAGAS[1], T. WORTHINGTON[1], B. YACOUBI KEYHANI[1], D. E. VAILLANCOURT[1,2,3,4], E. A. CHRISTOU[1,3,4];
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Abstract: Parkinson's Disease (PD) is a prevalent neurodegenerative movement disorder that primarily affects basal ganglia, a group of brain nuclei essential for the initiation and regulation of movements, procedural learning, and the automation of acquired motor skills. Despite this understanding, many questions remain about the features of motor learning deficits in PD, specifically in retaining and recalling acquired motor skills over time without additional practice (motor retention). In this 2-year longitudinal study, we utilized an established open-loop goal-directed force task involving the index finger and ankle to investigate if motor retention is impaired in individuals with PD. Our aim was to determine whether endpoint accuracy—quantified by force and temporal errors—deteriorates significantly over two years in PD compared with healthy controls (HC). Participants visited twice over two years and performed an index finger abduction task (31 PD, 18 HC) and an ankle dorsiflexion task (29 PD, 16 HC). The goal-directed force task required participants to match the peak force to a target at 15% of maximum force within 160 milliseconds. After two years, both force and temporal errors in the index finger task exacerbated for PD but not for HC. Specifically, force errors significantly increased in PD (22.6 ± 40.0%) but decreased in HC (-15.7 ± 24.6%). Temporal errors significantly increased in PD (23.1 ± 56.6%) but remained about the same in HC (4.6 ± 54.0%). In the ankle task, only temporal errors significantly exacerbated for PD (48.9 ± 84.8%) but not for HC (9.9 ± 52.5%). Furthermore, PD with greater disease severity levels (UPDRS-III) exhibited greater temporal errors after two years for index finger abduction task, as evidenced by a significant correlation between changes in disease severity levels and temporal errors ($r=0.51$, $p<0.01$). This study provides novel evidence that long-term motor retention is impaired in PD

but not in HC. The impairments appear to be more robust for temporal characteristics, as they are evident in both upper and lower limb movements.

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Poster

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Program #/Poster #: PSTR077.06/O5

Topic: F.04. Voluntary Movements

Support: NIH R01 NS133403-01

Title: The critical period of premotor cortex reorganization necessary for spontaneous motor recovery following injury to primary motor cortex

Authors: *H. M. HUDSON[1], L. S. HILD[1,2], H. E. CLEMENT[1], D. J. GUGGENMOS[1]; [1]Physical Med. and Rehabil., Univ. of Kansas Med. Ctr., Kansas City, KS; [2]Psychology, Univ. of Kansas, Lawrence, KS

Abstract: Acquired brain injuries such as stroke impact over 800,000 people annually in the United States and can leave survivors with severe impairments and very limited treatment options. When an injury occurs in primary motor cortex (M1), there is a global disruption of motor network activity (including corticocortical pathways and descending pathways to the spinal cord) leading to significant motor deficits. Despite the loss of M1, some spontaneous recovery of function can occur, although the precise location and mechanisms of recovery are largely unknown. One likely pathway is through neuroplastic reorganization within premotor cortex (PM), however, its role and timing in recovery is unclear. The purpose of this study is to determine if and when PM contributes to spontaneous motor recovery following M1 injury. Long-Evans rats (13 male, 17 female) were trained on a skilled reach task before random assignment to one of five groups. Rats were given an ischemic injury (or sham) over forelimb M1, while PM was inactivated using a constant infusion of muscimol, a GABA-A receptor agonist, for 0, 1, 2, or 3 weeks through an implanted cannula (Controls: C-injury - injury/no muscimol, C-muscimol - sham/3wk muscimol; Experimentals: E1 - injury/1wk muscimol, E2 - injury/2wk muscimol, E3 - injury/3wk muscimol). Skilled reaching data were collected during bi-weekly sessions for 8 weeks post-injury to assess the effect of PM inactivation on spontaneous motor recovery. A full-factorial ANOVA found a significant main effect of Behavioral Assessment Day ($p < 0.001$, $F_{(15,400)} = 103.35$) and an interaction effect of Group & Behavioral Assessment Day ($p < 0.001$, $F_{(60,400)} = 3.25$). These results indicate that PM inactivation significantly impairs task initiation and motor performance. Four days post-injury, all groups demonstrated a behavioral deficit compared to baseline performance with only C-injury

performing the reach task. Within one week following the cessation of muscimol infusion, all rats began reaching (except one rat in group E3). The C-injury group steadily improved from Day 4, reaching >80% baseline success by Week 5. All groups receiving muscimol reached >80% baseline success by 5 weeks post-muscimol cessation. These results suggest that PM is necessary to perform the skilled reach task, but that there is not a critical period (at least to 3 weeks post-injury) in which PM must be active after the injury for spontaneous recovery to occur. This may indicate that there is a longer post-injury window in which interventions could be effective.

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Program #/Poster #: PSTR077.07/O6

Topic: F.04. Voluntary Movements

Support: NIH T32 HD057850
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Title: Connectivity changes in spared ipsilesional cortical regions following ischemic injury of M1 in the rat

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Abstract: Stroke is a leading cause of motor disability in the world. Stroke leads to loss in neural communication within the injured cortical area and a disruption in global communication within spared brain regions. When an ischemic injury occurs within the sensorimotor system, there is a disruption in descending corticospinal pathways from primary motor cortex (M1) that gives rise to hemiparesis and other motor impairments. Additionally, there is a lack of signal integration within spared premotor (PM) and primary somatosensory (S1) areas which may contribute to the extent of motor injury. Functional reorganization is likely needed within and between spared PM and S1 if recovery is, in part, dependent on the reinstatement of sensorimotor integration. Understanding how changes in neuronal communication relates to recovery can help shape novel therapies for acquired brain injuries. The purpose of this study was to characterize how task-related connectivity of the PM-S1 network shifts following focal ischemic lesion to M1. Single-unit neuronal activity was recorded from Long-Evans rats via 32-channel microelectrode arrays

implanted ipsilesionally in PM and S1. Neuronal data was aligned to different reach phases of a skilled pellet-retrieval task. These recordings were collected prior to the induction of an ischemic injury to M1 and for one month following injury as motor recovery progressed. Graph theoretic techniques were used to analyze the efficiency in neuronal communication across PM-S1 networks defined by continuous-time transfer entropy of the underlying neurons' firing rates. We observed reductions in intra- and intercortical network connectivity following lesion induction. We also observed differences in PM and S1 responses to the lesion in both information flow and neuronal network architecture. Multilayer graph analysis and partial information decomposition showed how unique and synergistic source neurons contributed to information transfer during different reaching phases and M1 injury. These results suggest a dynamical reorganization of the sensorimotor network, reflecting shifts between coordinated sensorimotor integration and the selective routing of task-related information through distinct neuronal populations. All the connectivity metrics were strongly correlated with success rates on the reaching task. These results provide insight into the disruption of task-related sensorimotor communication induced by ischemic injury within M1.

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Poster

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#nextgenerationeu (ngeu) , project MNESYS (pe0000006)

Title: Stroke and Neural Dynamics: Exploring recovery from focal ischemic infarcts in the latent space

Authors: *F. BARBAN[1], M. NISHIMOTO[4], H. M. HUDSON[5], D. GIAMBARBARA[2], M. CHIAPPALONE[3], R. J. NUDO[6], D. J. GUGGENMOS[7];
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Abstract: Acquired brain injuries, such as stroke, are a leading cause of long-term disability worldwide. When injury occurs within the primary motor cortex (M1), descending signals to the spinal cord controlling movement and the integration of somatosensory and premotor inputs are disrupted, leading to motor deficits. Although spontaneous recovery can occur and is enhanced

by rehabilitative therapies, the underlying mechanisms are still under investigation. Recovery is likely supported by reorganization in spared premotor (PM) and somatosensory (S1) regions; understanding how these areas undergo neurophysiological changes following M1 injury is crucial.
Objective: To determine (1) if and how spontaneous recovery is aided by open- and closed-loop intracortical microstimulation, (2) whether neural population dynamics disrupted by M1 infarct are reconstituted within the sensorimotor network during recovery, and (3) whether between-area activity and integration are restored over 4 weeks.
Methods: Twelve Long-Evans rats were trained on a skilled pellet retrieval task and unilaterally implanted with chronic 32-channel microelectrode arrays in PM and S1. Neural data were recorded during task performance prior to and up to four weeks following a focal ischemic injury to M1. Gaussian Process Factor Analysis was used to estimate low-dimensional neural population trajectories, while Canonical Correlation Analysis quantified PM-S1 integration. Mean firing rate and local variation of refractoriness were also computed. Beginning on Day 9 post-infarct, rats received either PM spike-triggered (i.e., Activity Dependent Stimulation, ADS) or yoked, open-loop (OLS) stimulation in S1.
Results (Preliminary): The marked post-infarct disruption of task-associated latent manifolds in PM and S1 gradually reverted during recovery. By Week 2, these low-dimensional trajectories realigned, paralleling behavioral improvements in pellet retrieval. Task-associated neural modulation also gradually reappeared within and between monitored areas. Behaviorally, ADS animals achieved higher success rates, while OLS animals made more retrieval attempts.
Conclusions: Our findings suggest that recovery after M1 infarct is accompanied by re-emergence of a task-associated manifold in spared PM and S1 and stepwise restoration of cross-area coordination. ADS may further expedite behavioral improvement and network-level recovery, whereas OLS yields more modest gains.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR077.09/O8

Topic: F.04. Voluntary Movements

Support: NIH/NINDS R00NS092972
NIH/NINDS R01NS122969
NIH/NINDS R21NS135361
Boston University Center for Systems Neuroscience Distinguished Fellowship
Whitehall Research Foundation

Title: Using high-density electrodes to investigate laminar dynamics in premotor cortex during grasping

Authors: ***N. CARR**[1,2], A. L. SMOULDER[3], T. ARORA[4], T. WANG[2], K. LEE[5], K. R. MARMON[6], J. SALAMY[7], C. CHANDRASEKARAN[6,2,5,3];
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Abstract: A large body of research has used Utah arrays to understand dynamics during reaching and grasping parallel to the cortical surface of motor and premotor cortex. However, we currently lack an understanding of coordinated neural circuit dynamics across layers of these motor areas. We addressed this open question using high-resolution Neuropixels 1.0 NHP electrodes (45 mm shank with 20 micron spacing) to record from multiple layers of the dorsal premotor cortex (PMd) and the underlying white matter, in two monkeys (T and Y, n= 9 sessions) during a grasping task.
We adapted an existing turntable design to present objects for a delayed reach-grasp-lift task. In this task, a button with a light turns on, cueing the animal to initiate the trial, touching and holding for a variable delay period. At the end of the delay period, the animal is cued to reach, grasp, and lift an object. We recorded video of the hand from multiple angles and used Jarvis Annotation Tool and DeepLabCut for markerless tracking of the grasp kinematics (see Arora et al.). Monkey T performed a simple precision grasping task, whereas monkey Y grasped spheres varying in weight, with weight indicated by color. Behaviorally, we found that movement time (the time between movement onset and object lift), increased monotonically with the weight of the object.
We first examined if the spectrolaminar profile in PMd is consistent with the spectrolaminar profile of visual and prefrontal areas, with stronger beta band activity in deep layers and stronger gamma band activity in superficial layers. Preliminary analysis in both monkeys suggests a spectrolaminar profile with some differences from this canonical motif. We observed a peak in low beta frequencies (12-20 Hz) in the deeper channels, and a peak in higher beta frequencies (20-35 Hz) in the superficial channels. Moreover, the pattern of gamma band activity (75-150 Hz) was different in monkeys T and Y, possibly due to anatomical differences between recording areas within PMd.
We found that in both tasks, PMd showed a large change in activity preceding movement onset. However in the weight manipulation task, neural encoding of object weight only emerged after movement onset. While superficial and deep neurons exhibit highly similar population-level dynamics after movement onset, we find that deep neurons exhibit object weight encoding (and likely related behavioral differences) earlier than superficial layers.
Ongoing experiments are focused on additional Neuropixels recordings along the rostral-caudal axis of PMd, combined with analysis of dynamics in different layers during these motor tasks to understand laminar microcircuit dynamics in the PMd during grasping.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Topic: F.04. Voluntary Movements

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NIH NINDS R21NS135361

Title: Separable interactions between premotor and motor cortex during reach to grasp

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Abstract: When grabbing an object, we reach while preparing and executing a grasp. It remains unclear how distinct streams of action-relevant information (where to reach and how to shape the hand) are shared across brain areas. To investigate, we trained a rhesus macaque to perform a delayed reach-to-grasp task, manipulating reach direction (left/right) and object size (small/large), while simultaneously recording from dorsal premotor (PMd), ventral premotor (PMv), and primary motor cortex (M1) with Utah arrays.

We considered three hypotheses of how direction and size information flow in this microcircuit. At one extreme, all task-relevant information may appear in all areas simultaneously as soon as it becomes available ("everywhere" hypothesis). It could also be that there are differences in timing across areas, but once information is shared between two regions, all streams (direction and size) are indiscriminately shared together ("indiscriminate" hypothesis). Lastly, specific types of information may be shared selectively across areas with distinct communication patterns ("selective" hypothesis).

To arbitrate among these, we examined the timing of direction and size information in each area via decoding accuracy. PMd and M1 were tightly aligned when decoding reach direction, peaking near movement onset. In contrast, PMv lagged in direction decodability by ~175 ms. When decoding object size, PMd peaked before movement, whereas PMv and M1 did so later, near the time of object contact. Thus, PMd and M1 shared reach (direction) information in time but not grasp; M1's grasp (size) information was more tightly coupled with PMv.

We next computed residual correlations between single-trial decoder projections between areas, reasoning that these reflect shared fluctuations along task-informative dimensions of neural activity. PMd-M1 showed peaks in location coding correlation before and during reach; PMv-M1 followed a similar pattern with a ~160 ms lag. For size, PMv-M1 correlations rose before movement and then declined; PMd-M1 showed a similar trend with a ~120 ms lag. These results indicate that PMd and M1 couple earlier along reach-relevant dimensions, while PMv and M1 couple earlier for grasp.

Our findings so far support the "selective" hypothesis: while PMd, PMv, and M1 all encode reach and grasp information, the timing and content of inter-area communication reflect

structured differences aligned with specialized roles. This aligns with anatomical studies that emphasize the role of PMd in proximal limb movements and PMd in distal. Ongoing work seeks to further detail the timing of information-specific population level signals shared between areas.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Title: Towards 3d markerless pose tracking during reach to grasp in macaque monkeys

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Abstract: A reach to grasp is a complex motor act that requires coordinating multiple joints in 3D that has allowed primates to develop and use tools. Precise quantification of grasping behavior presents a barrier in understanding of the underlying neural dynamics. Current state-of-the-art setups use invasive markers or gloves for 3D hand tracking which can impede natural movements, or are difficult to keep on monkeys' hands while they are doing these tasks. Hence, markerless pose tracking is an essential first step. Here, we demonstrate a reliable solution that combines multi-camera acquisition with deep learning, 3D pose markers, and triangulation for markerless pose tracking and estimation in 3D.
For acquisition, we use 4 Blackfly FLIR cameras triggered and acquired through the JARVIS Acquisition System at 50 FPS while monkeys perform a reach-to-grasp task. For pose estimation, we use the following steps: data recording and frame annotation using JARVIS, frame prediction using DeepLabCut, and triangulation to 3D space using openCV. We typically annotate 8-10 frames per trial for 20-30 trials across 4 cameras. Then, using this training data we train a Resnet101 model in DeepLabCut (DLC) to track the keypoints on the wrist and the hand for all cameras. We measured the robustness of single-camera 2D tracking in two ways. We first examined if the velocity profiles for markers (e.g., thumb tip) across trials were stereotyped

when aligned to the stop button, the light curtain break or the time of object lift. Peak velocity was reasonably stereotyped and aligned with light break, and object lift time with trial average delay of 60ms. Second, we also examined if our estimated hand aperture tracked the size of the presented object. We found that for a cube 2.5 times the size of a smaller cube the hand aperture increased by a factor of 2.03.
We considered multiple approaches for marker tracking and triangulation in 3D, such as utilizing JARVIS' pre-built hand model via HybridNet, or Anipose in tandem with DLC models. However, we were unable to yield reliable model fitting and triangulation using these methods. Thus, we combined the strengths of DLC's single camera tracking models and JARVIS' 3D annotation tool. We trained the DeepLabCut models on 3D annotations from JARVIS. However, our camera setup has staggered exposure times for synchronization (3 ms for the fastest, to 12 ms for the slowest) causing inconsistency between annotations and predictions for cameras with longer exposure times for frames with fast hand motion. This makes 3D triangulation challenging when the animal rapidly initiates the action. Ongoing efforts aim to improve the reliability of the triangulation and 3D pose estimates.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Topic: F.04. Voluntary Movements

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Title: Choking under pressure: overcompensating for arousal?

Authors: *M. MCDONNELL[1], A. CHANDRASEKARAN[4], C. KI[2], A. L. SMOULDER[5], B. M. YU[1], A. P. BATISTA[6], M. A. SMITH[3], S. M. CHASE[2];
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Abstract: Performance tends to improve when a more substantial reward is at stake. However, there can be a paradoxical decrease in performance when rare and large ("jackpot") rewards are offered, which is known as "choking under pressure". But what causes choking under pressure?

One class of explanations suggests an arousal response which interferes with behavior. But some behavioral metrics of choking indicate it comes from an excess of self-monitoring, as indicated by overly cautious strategies. Here we report evidence for a mixture of the two explanations: a heightened arousal state in conjunction with an increase in cautious tendencies. We trained three rhesus monkeys to perform a challenging visual change detection task, where they reported the change by making a precise movement (joystick or saccade) to a target. We cued the monkeys as to what reward was available on every trial: on 95% of trials the reward was modestly sized, while on the remaining 5% of trials a rare jackpot reward (10x the average size of the others) was offered. All three animals choked under pressure, in that they underperformed for these jackpot rewards. To shed light on the underlying causes of choking under pressure, we investigated two broad categories of metrics: those relating to arousal state, and those relating to self-monitoring. To assess the monkeys' arousal state, we measured their pupil size and the rate of small fixational eye movements (microsaccades) during the waiting period of the task (i.e., before any movement). We found that both of these metrics increased with greater rewards, suggesting a monotonic relationship between reward and arousal state even for jackpot trials. To assess self-monitoring, we looked for signs of the animals' caution during the task by measuring the speed of their movements. Interestingly, we found that both their peak movement speed to the target as well as their reaction times to initiate movement were both slowed for jackpot rewards compared to the other rewards. Together, these results indicate that the monkeys were more cautious for larger rewards. In summary, jackpot rewards seem to impact multiple aspects of the monkeys' behavior. Before they even moved, we saw evidence of increased arousal. During movement, we saw evidence of increased caution. Arousal and caution are both reasonable internal responses to an increased reward, but it is possible that monkeys choked under pressure by errantly overcompensating for an increased arousal state by over-monitoring and employing an overly cautious strategy.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

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Topic: F.04. Voluntary Movements

Support: Grant-in-Aid for Scientific Research (B) (24K02840) from Japanese Society for Promoting Science

Title: Mapping the cortical representation of upper limb muscle synergies via simultaneous EMG and fMRI recording

Authors: *H. HIGASHII[1], A. OSHIMA[2], K. ISHIKAWA[4], N. KANEKO[5], T. KATO[6], K. NAKAGAWA[7], K. NAKAZAWA[3], K. TAKIYAMA[8], H. YOKOYAMA[9];

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Abstract: The human central nervous system (CNS) is thought to generate coordinated motor output through combinations of motor modules known as muscle synergies. Previous studies have shown that a small number of basis vectors (muscle synergies) extracted from electromyographic (EMG) signals can account for most muscle activity; however, the neural basis of these synergies remains poorly understood. Understanding the structure of muscle control would not only deepen basic neuroscience knowledge but also contribute to clinical insights into motor impairments following stroke or spinal cord injury. This study investigated the neural representation of coordinated muscle control by simultaneously recording brain activity using functional magnetic resonance imaging (fMRI) and muscle activity using surface EMG. Eight healthy young adults participated in the study. Participants performed an isometric force task with their right upper limb inside a 3T MRI scanner, during which brain activity was recorded using fMRI. To complete the task, they exerted force to control a cursor, moving it toward one of eight target directions displayed on a screen. Surface EMG was recorded from 12 muscles involved in the movement. As a control task, participants performed a wrist extension to investigate differences in cortical control of the wrist extensor muscle when acting independently versus in coordination with other joint muscles. The EMG data were preprocessed and decomposed into muscle synergies using non-negative matrix factorization (NMF). The time course of each synergy was convolved with a canonical hemodynamic response function (HRF) and used as a regressor in a general linear model (GLM). fMRI data were analyzed using SPM12. Brain activity was first estimated at the individual level, followed by group-level (second-level) analysis. All participants showed 3-4 extracted muscle synergies, which were primarily categorized into flexor- and extensor-dominant groups. BOLD signal changes associated with each synergy were observed mainly in the motor cortex. Notably, peak activations were distributed along the anterior-posterior axis depending on the specific synergy, suggesting a functional segregation within the motor area. In addition, the area related to the wrist extension task was distinct from the areas related to the muscle synergies, even though both involve the same wrist extensor muscle. These findings provide novel evidence that muscle synergies correspond to spatially segregated regions in the motor cortex, supporting the existence of areas that play a role in modular motor control in the human brain.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: PSTR077.14/O13

Topic: F.04. Voluntary Movements

Support: NSERC
Connected Minds Program, supported in part by CFREF.

Title: Task-specific neural signatures of grasping and placing: an EEG study

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Abstract: Successful interactions with our environment rely on coordinated movements, such as grasping and placing objects, yet most research has focused on object acquisition, overlooking the relocation phase. While both actions share common features, such as hand localization, orientation, and sensitivity to task complexity, they differ in sensory reliance and movement intention. Grasping is typically guided by visual input and concludes with haptic feedback at contact, whereas placement often depends on an internal representation of the target location. To investigate the neural mechanisms supporting these distinct movement goals, EEG data were recorded from 31 right-handed young adults (mean age = 22 years, SD = ±2.4) as they either grasped an object or placed it onto a shape- and size-matched placeholder. We hypothesized task-dependent differences in source-localized brain activity during both the early sensory encoding and later motor preparation phases, prior to movement onset. Time- and frequency-domain source localization analyses revealed distinct neural patterns between the two tasks. In the time domain, we observed significantly increased parieto-temporal activity during early sensory exposure to the object-to-be-grasped compared to the placeholder, suggesting task-specific sensory processing. This was retained in the motor preparation phase, extending towards motor-related areas. In the frequency domain, early sensory processing for placing revealed significant gamma-band (28-40 Hz) desynchronization over parietal areas, followed by increased desynchronization of frontal and motor areas over the alpha and beta frequency bands during the motor preparation phase, relative to grasping. Together, these findings highlight task-specific neural dynamics that reflect movement intention before execution. The distinct spatial and temporal activity patterns suggest differential engagement of sensory-motor pathways during planning of grasping vs. placing. These insights will enhance our understanding of motor control and support the development of real-time movement classification systems for assistive technologies.This research is supported by funding from NSERC and the Connected Minds Program, supported in part by CFREF.

Disclosures: P. Georgiadis: Other; Center for Integrative Neuroscience, York University, Toronto, ON, Canada, Center for Vision Research, York University, Toronto, ON, Canada, Department of Biology, York University, Toronto, ON, Canada. **E. Freud:** Other; Center for Integrative Neuroscience, York University, Toronto, ON, Canada, Center for Vision Research, York University, Toronto, ON, Canada, Department of Psychology, York University, Toronto, ON, Canada, Department of Biology, York University, Toronto, ON, Canada. **P. Kohler:** Other; Center for Integrative Neuroscience, York University, Toronto, ON, Canada, Center for Vision Research, York University, Toronto, ON, Canada, Department of Psychology, York University, Toronto, ON, Canada. **J. Crawford:** Other; Center for Integrative Neuroscience, York

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR077.15/O14

Topic: F.04. Voluntary Movements

Title: Identifying discontinuities in the somatotopy of motor cortex in non-human primates

Authors: *E. FITZGERALD[1], T. MADISON[2], R. MCCOLLUM[2], L. JOHNSON[4], J. ZIMMERMANN[3], D. FAIR[2];
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Abstract: Since the 1930s, the predominant view has been that primary motor cortex (M1) is somatotopically organized, meaning that each body part (effector) corresponds to a cortical location. A recent study identified a discontinuity in the somatotopic organization of primary motor cortex (M1), revealing regions situated between the arm and leg (effector) regions that respond to movements involving multiple effectors. Termed inter-effector regions, these areas exhibit increased activation both before movement initiation and during multi-effector actions such as walking, suggesting a role in planning complex, coordinated movements. Moreover, inter-effector regions show strong functional connectivity with supplementary motor cortex (SMA), striatum, cerebellum, and sensorimotor regions of the thalamus. Collectively, these interconnected areas form a network called the somato-cognitive action network (SCAN). In this study, we aimed to identify inter-effector regions in non-human primates (NHP). To do this, five NHPs were scanned at rest using ultra-high field (10.5T) functional magnetic resonance imaging (rsfMRI). We identified a similar discontinuity in the motor somatotopy in three of the NHPs. Additionally, we found that inter-effector regions identified in our NHPs had similar patterns of connectivity with the SCAN network (increased connectivity in basal ganglia and sensorimotor thalamic nuclei). In conclusion, this study highlights that the SCAN network is present across humans and NHPs, which could open opportunities for further investigation of how inter-effector regions are involved in motor processes.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Program #/Poster #: PSTR077.16/O15

Topic: F.04. Voluntary Movements

Support: Cortivision

Title: Brain hemodynamic responses to a motor control paradigm in younger and older adults, and people with Parkinson's disease: An fNIRS mediated feasibility study

Authors: *M. BJALME-EVANS;
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Abstract: Objective: This study aims to investigate the use of fNIRS in measuring the hemodynamic response of the brain during motor control tasks of increasing complexity in people with Parkinson disease (PwP) and healthy younger and older adults. Background: Parkinson disease (PD) is a neurodegenerative disease that presents with bradykinesia, rigidity, postural instability, and resting tremor. Motor learning and control are also affected in PwP; however, studies investigating the neural correlates of these deficits are often not informed by motor control theory. Gentile's 2-D taxonomy is a motor control paradigm that systematically increases complexity, which may be used to create a more nuanced protocol compared to single vs. dual-tasking. Methods: We will use Gentile's 2-D taxonomy to devise an experimental session based on motor control theory. Participants (15 PwP, Hoehn & Yahr I-III (50-85 years old); 15 older adults (OA) (50-85 years old); and 15 young adults (YA) (18-30 years old)) will perform dynamic and static balance tasks at increasing levels of complexity while wearing a Cortivision Spectrum C23 near-infrared spectroscopy (NIRS) device (16x16 montage). Participants will also wear APDM Opal Sensors to evaluate aspects of movement, such as velocity, stride length, and sway. Results: We aim to analyze the hemodynamic response differences of the frontal cortices between groups, as well as the interaction of group x task complexity level using repeated measures ANOVA. We predict that PwP will have an increased hemodynamic response compared to OA and YA, especially with increasing task complexity. We also aim to validate fNIRS using APDM Opal sensors to compare if changes in movement patterns are reflected in the hemodynamic response as measured by the NIRS device with a similar sensitivity. Conclusions: This study will be the first to validate functional NIRS (fNIRS) against APDM. We will also be the first to use a paradigm well established motor control theory in order to systematically increase complexity during an fNIRS mediated study. If results reach statistical significance, and are validated against APDM, this is a further step toward using fNIRS as a non-invasive and cost-effective outcome measurement tool in PD.

Disclosures: M. Bjalme-Evans: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cortivision.

Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Topic: F.04. Voluntary Movements

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Title: Motor experience causally shapes the functional organization of motor and language networks

Authors: *Y. LIU[1,2], J. SUN[2], L. CHEN[3], L. WANG[2];
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Abstract: Cerebral functions show asymmetry and lateralization. While widely considered to be genetically driven, whether functional lateralization exhibits experience-dependent plasticity is largely unknown. Meanwhile, the causal dependence in lateralization between functions is scarcely studied. Language and action are domains with salient lateralization, both showing left-hemisphere dominance in frontal and parietal association areas, coinciding with the prevalence of right-handedness. Interestingly, handedness statistically correlates with language lateralization, with a weaker left lateralization in left-handedness. Are handedness, motor, and language lateralization parallelly arranged by genetic factors, or alternatively, are motor and language lateralization driven by motor experience of hand use? We address these questions in individuals whose brachial plexus, peripheral nerves that control upper limb movement, were injured during birth (obstetric brachial plexus injury, OBPI), resulting in the intact hand being dominant. This population thus allows us to examine the causal influence of postnatal motor experience on motor and language organization in the brain. Participants performed a motor (hand grasping) and a language (sentence comprehension) task in an fMRI scanner. In participants with right OBPI who were forced to be left-handed, we found clear evidence of right-hemisphere motor dominance. First, stronger activity was found in right vs. left dorsal premotor cortex (PMd), superior lobule, and supramarginal gyrus (SMG), in contrast to righthanded controls. Second, in premotor cortex and intraparietal sulcus, there were more voxels in the right vs. left hemisphere that responded to either hand or jointly activated by both hands. These patterns contrast with the contralateral pattern in primary sensorimotor and secondary somatosensory cortices. Crucially, decreased left-hemisphere dominance in language was observed in frontal language networks, including middle frontal gyrus and SMG, and a right-hemisphere dominance in the anterior middle temporal lobe, compared to controls and the left OBPI group. Therefore, although the brain was preset to be left lateralized, postnatal left-handedness shifted motor dominance to the right hemisphere and, importantly, reduced left lateralization in language. These findings provide novel evidence for experience-driven motor

and language organization and, for the first time, reveal causal dependence of language networks on motor experience, shedding light on the development of these systems in the human brain.

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Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Title: Cortical Control of Grasping vs. Placing Objects: an fMRI Study

Authors: *G. N. LUABEYA[1], E. FREUD[2], J. CRAWFORD[3];
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Abstract: Studies of hand control tend to focus on reaching and grasping an object, but often ignore what follows: often placing that object in a new location and orientation. In this study, we used a neuroimaging technique to explore the neural correlates of object placement and how it differs from grasping. We employed an event-related fMRI task in which twenty participants alternated between grasping and placing a rectangular object supported on a template on an inclined platform. Each trial began with a target presentation (illumination of the object or an empty template), followed by a planning delay period before receiving a cue to perform the required movement (either grasp or place) and return to the home position on their pelvis. The object / template was presented at two locations (left / right of central fixation) and two orientations (clockwise / counterclockwise), randomized and controlled from behind by sliding / rotating the template across the platform. Univariate analysis revealed increased activity in the frontal cortex, the insula, the cingulate cortex, the superior temporal gyrus, striate cortex during planning of either movement. Task-specific increases in activation were observed in the right extrastriate region and the left superior temporal sulcus in preparation for a grasp, vs. right dorsolateral prefrontal cortex, left primary motor, and left primary somatosensory cortex in preparation for placement. During the execution phase, both tasks produced activity in the insula, the middle and posterior cingulate cortex, the superior temporal gyrus, and the striate cortex, with task-specific increases in activation in the left supplementary motor area, left anterior prefrontal cortex, and the left extrastriate cortex during grasp, and the right extrastriate region in preparation for placement. An ROI analysis showed significantly higher activation for placement in left anterior intraparietal cortex and primary somatosensory cortex during planning, and posterior intraparietal cortex and the primary somatosensory cortex during execution. These

findings suggest that grasp and placement share a number of overlapping cortical mechanisms, but also show regional specialization, likely related to different intent and sensory feedback.

Disclosures: G.N. Luabeya: None. **E. Freud:** None. **J. Crawford:** None.

Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR077.19/P2

Topic: F.04. Voluntary Movements

Support: NIH NINDS under Award Number R01NS131227 (PI: Vahdat)
Florida Department of Health James and Esther King Biomedical Research, Award Number 23K07 (PI:Vahdat)

Title: Mapping Medullary and C3-C4 Propriospinal Pathways Underlying Mammalian Forelimb Force Control

Authors: V. JINDAL[1], M. M. GRUDNY[2], D. W. WESSON[4], D. E. VAILLANCOURT[3], ***S. VAHDAT**[5];
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Abstract: Our classic view of neural control of movement states that the execution of goal-directed upper limb movements mainly relies on the activation of cortical motor areas. On the other hand, postural and balance control heavily relies on the activation of brainstem motor nuclei. However, evidence from animal work is starting to challenge this view by demonstrating active involvement of multiple brainstem and intersegmental spinal cord circuits in the execution of goal-directed forelimb movements, including the medullary reticular formation (MRF) and the propriospinal neurons at C3-C4 cervical levels. Yet we do not know whether these findings extend to humans with a more developed corticospinal tract.
Here, we characterized the brain-wide functional organization of medullary regions involved in the regulation of forelimb force by conducting functional magnetic resonance imaging experiments in humans and mice performing a unilateral hand force control task. In humans, we used simultaneous spinal cord-brain fMRI to assess brain and cervical cord activations and functional connectivity. In mice, we developed a novel system to acquire awake fMRI data, while thirsty head-fixed mice performed a forepaw force control task in an 11T Bruker scanner. We identified two medullary regions, caudal medulla (CaudM), and lateral rostral medulla (Lat-RM) that are specifically associated with the regulation of hand/forepaw force. Across species, the sensorimotor cortex displayed the strongest functional connectivity with CauM, followed by Lat-RM, and then the more medially located medial rostral medulla (M-RM). In mice, the cortico-medullary coupling in both CaudM and Lat-RM increased systematically along a ventro-

medio-dorsal gradient, with the strongest links to primary motor and premotor cortices. In humans, by contrast, higher-order sensorimotor regions (anterior cingulate, insula, and second somatosensory cortex) drove the strongest connectivity with CauM and lateral Lat-RM, while M-RM remained weakly engaged.
Furthermore, we identified significant activation at the C3-C4 cervical level related to hand force control in humans. Our results revealed distinct functional territories within the C3-C4 cervical level, with ventral regions showing strong connectivity to the medulla and dorsal regions linking to lower cervical levels in humans.
Our findings indicate the presence of an indirect pathway involving both the reticulospinal pathway and the C3-C4 propriospinal system, which actively contributes to fine hand force control in the mammalian brain.

Disclosures: V. Jindal: None. **M.M. Grudny:** None. **D.W. Wesson:** None. **D.E. Vaillancourt:** None. **S. Vahdat:** None.

Poster

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Program #/Poster #: PSTR077.20/P3

Topic: F.04. Voluntary Movements

Support: NINDS Grant R01 NS114046

Title: Cortical activity pattern after peripheral nerve repair is unrelated to surgical type or functional hand use: a representational similarity analysis

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Abstract: Nerve repair and transfer surgery offer promising methods to restore function after peripheral nerve injury in the upper extremity (PNI), but it remains unknown whether post-surgical functional recovery depends on patterns of neural activity. Neural activity patterns should be particularly important after nerve transfer surgery, as rerouting of peripheral nerves should require cortical reorganization to support functional recovery. Functional outcomes after nerve surgery are inconsistent, so a better understanding of its neural mechanisms may lead to improved interventions. Here we compared the functional relevance of movement-related activity patterns in primary motor cortex (M1) in individuals who received nerve transfer after PNI, those who received other (non-transfer) surgery after PNI, and healthy controls. We hypothesized that patients with greater functional use of the affected hand would exhibit M1 activity patterns more similar to healthy controls; and that nerve transfer would lead to distinct patterns of neural activity. **Methods:** 36 right handed adults (24 healthy controls; 12 patients with right upper limb PNI: 3 with transfer surgery, 9 with non-transfer surgery) performed a

precision drawing task using the right hand during fMRI, and a block building task that measured the pattern of right/left hand choices in a goal-oriented reaching task. Inter-subject representational similarity analysis was used to compare neural data (voxel-wise BOLD activity in M1 contralateral to movement) and behavioral data (functional use of right hand). For each dataset (neural, behavioral), a dissimilarity matrix was computed to capture between-subject differences, and correlations between the two matrices were measured with the Mantel test. We also applied hierarchical clustering to each dissimilarity matrix to identify group membership. **Results:** Neither our neural data nor our behavioral data clustered by participant type; the 3 nerve transfer patients were assigned to different clusters in both neural (4 clusters) and behavioral (3 clusters) data. The Mantel test revealed no significant correlation between the neural and behavioral matrices ($p = 0.14$).**Conclusion:** The absence of association between the neural and behavioral matrices suggests that individual differences in functional hand use are not driven by M1 activation patterns. Instead, the neural mechanisms for functional hand use may be more complex, distributed, and/or non-M1. We found no evidence that nerve rerouting leads to cortical reorganization; instead, M1 representations were highly individualized, likely driven more by task performance than by clinical status.

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Poster

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Program #/Poster #: PSTR077.21/P4

Topic: F.04. Voluntary Movements

Support: KAKEN JP23K10782
KAKEN JP23K27958

Title: Estimating ability to develop motor skills by deep learning-assisted analyses of resting-state brain activity

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Abstract: Motor learning plays fundamental roles in daily life by enabling the acquisition of a wide range of physical skills. However, the rate at which individuals learn new motor skills can vary considerably. Understanding the neural basis underlying this variability remains a key objective in neuroscience. We investigated whether resting-state brain activity measured prior to motor training could predict subsequent performance in learning the novel motor task of juggling. Sixty-eight healthy, right-handed adults with no prior juggling experience participated

in this study (forty-four men and twenty-four women). We first measured resting-state brain activity for approximately 10 min using functional magnetic resonance imaging (fMRI). Following their scan, each participant moved to another room to perform a motor-learning task involving juggling two balls with their right hand in a continuous rolling motion. Resting-state fMRI data were preprocessed using SPM12. We then calculated the mean fractional amplitude of low-frequency fluctuations (mfALFF). The total number of catches during the juggling task was used as an index of motor learning ability. Participants were divided into two groups based on their performance: the 34 high performers and the 34 low performers. We employed a two-dimensional convolutional neural network (2D-CNN) to classify participants into high and low performers using their mfALFF brain images as input features. The model achieved a classification accuracy of 82.73% ($p < 0.01$). This finding indicates that resting-state brain activity, even before any motor learning takes place, contains informative patterns predictive of future motor learning success and suggests that individual differences in motor learning ability may be linked to spontaneous neural dynamics observable at rest. Our findings suggest that resting-state brain activity reflects current cognitive states and may also provide predictive insights into future behavioral outcomes.

Disclosures: H. Kadota: None. **R. Yamaguchi:** None. **H. Sekiguchi:** None.

Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

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Program #/Poster #: PSTR077.22/P5

Topic: F.04. Voluntary Movements

Support: NSF GRFP Fellowship

Title: Modeling cortical-subcortical network interactions between electric fields and stroke lesions in non-human primates

Authors: *I. OKIDHAIN[1], P. KHANNA[2], S. M. GRIFFIN[3], H. CHOI[4], K. GANGULY[4]; [1]Univ. of California, Berkeley, BERKELEY, CA; [2]Neurosci. & EECS, UC Berkeley, Berkeley, CA; [3]Helen Wills Neurosci. Inst., [4]Neurol., UCSF, San Francisco, CA

Abstract: Alternating current stimulation (ACS) is a promising motor rehabilitation therapy for stroke patients. While certain aspects related to dosimetry, such as frequency dependence, have been investigated in detail, the engagement of cortical-subcortical networks remains unclear. We addressed this gap by simulating the electric fields of six non-human primates (NHPs; rhesus macaque) from epidural ACS studies and analyzing both the cortical and subcortical recruitment at the average, maximum, and minimum E-field values. Using electrode configurations and stimulation parameters analogous to those used in prior studies (Khanna et al., 2021; Griffin et al., 2025), we simulated the induced E-field distributions and directions via a finite element model (FEM) comprising 46 biological materials of the head, the intact craniotomy, and the neck

tissues. In past epidural ACS experiments, implanted 64-channel sensory electrode arrays located in the primary motor cortex (M1) exhibited average E-field magnitudes of 2.13, 2.92, and 4.49 V/m, as measured at the stimulation frequency peaks and troughs (Khanna et al., 2021). We determined that the maximum E-field magnitudes at the stimulating electrodes were higher than the magnitudes at the sensory electrodes. Next, we segmented the E-field FEMs to identify activated regions in both the cortex and subcortex. We then computed the E-field direction between the stimulation electrodes to determine the manner in which the E-field was distributed across the brain regions. Based on the parcellated FEMs, we predicted that the epidural ACS recruited not only cortical motor areas like the M1, but also multiple subcortical regions, such as the caudate. However, the E-field values simulated for the subcortex were lower than those measured at the M1 sensory electrode array. The M1 post hoc E-field value was successfully employed to validate the FEM E-field value in the same brain region. In addition, to verify the subcortical region predictions, we are in the process of validating depth-dependent field penetration by inserting electrodes at varying cortical depths in an ex vivo rhesus macaque brain, then computing the E-field values at the locations of sensory electrodes implanted in the six NHPs, as in Khanna et al., 2021. In conclusion, our study demonstrated that the epidural ACS E-field engaged both cortical and subcortical motor regions. Subcortical regions like the putamen, involved in the regulation of motor movements, are not normally included in ACS studies. However, a more robust understanding of how subcortical regions respond to stimulation may improve the specificity of post-stroke motor recovery interventions.

Disclosures: I. OKidhain: None. **P. Khanna:** None. **S.M. Griffin:** None. **H. Choi:** None. **K. Ganguly:** None.

Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

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Program #/Poster #: PSTR077.23/P6

Topic: F.04. Voluntary Movements

Support: TWCF-2022-30259

Title: Acting without consciousness: Movements require awareness, muscle activations do not

Authors: *T. DOMINIK[1], A. BUCCELLA[2], J. WIDI[1], T. LAN[1], L. MUDRIK[3], U. MAOZ[1], A. SCHURGER[1];
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Abstract: The neuroscience of consciousness has largely focused on perceptual—typically visual—awareness, with comparatively less attention to the role of consciousness in action initiation. In the context of cued action, two types of awareness are relevant: awareness of the stimulus and awareness of one's own movement. In this study, we investigated whether

stimulus-triggered movements can be initiated without movement awareness and, if so, which characteristics of movement its consciousness depends on.
Fifty participants completed a simple go/no-go task (3:1 ratio), responding to go stimuli with a button press. Each trial included simultaneous recording of electromyographic (EMG) activity, finger position via a high-precision motion tracker, and finger acceleration via an accelerometer. On each successful no-go trial, participants were asked whether they were aware of having moved at all, no matter how small the movement was. Participants were specifically trained to adopt a highly liberal reporting criterion—responding 'Yes, I moved' even with the slightest doubt. Thus, 'No' responses indicated high confidence in the absence of any perceived movement.
The results showed that in trials where participants reported no perceived movement, no overt movement was detected via position tracking or accelerometry. However, EMG data occasionally revealed brief, low-amplitude bursts of muscle activity even in the absence of action awareness. We compared these EMG patterns to an earlier baseline condition where participants passively viewed the same stimuli without knowing that one would later serve as a go cue. EMG bursts were virtually absent in this baseline condition, suggesting that the non-conscious EMG activations were specific to the movement cue in the go/no-go task.
The presence of cue-related EMG activations of which participants were not aware suggests that neuromuscular activity alone is not sufficient for conscious awareness of a motor response. Importantly, these non-conscious activations were also insufficient to produce overt movement: when overt movement was detected, it was reliably reported. This indicates that the boundary between conscious and non-conscious movement may depend on mechanical muscle contraction and/or proprioceptive feedback. These findings have implications for current theories of consciousness, which must explain why stimulus-cued electrical activity in the muscles can remain unconscious while overt muscle contractions do not.

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Poster

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Topic: F.04. Voluntary Movements

Support: NINDS Grant NS125141

Title: Inhibition of Anterolateral Motor Cortex Leads to Specific Reduction of Compulsive Self-Grooming

Authors: *G. G. LIU[1], S. E. AHMARI[2];
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Abstract: Maladaptive behavioral sequencing is observed in obsessive-compulsive disorder (OCD) and obsessive compulsive-related disorders (OCRD), which are characterized by repetitive and perseverative thoughts and actions. Functional neuroimaging studies have consistently identified alterations in corticostriatal circuits in people with OCD both at baseline and during symptom provocation; however, the neural substrates of impaired sequencing are less explored. The *Sapap3* knockout (KO) mouse model, which displays robust perseverative grooming behavior, can be used to dissect the circuits underlying this important phenotype. Previous work in our lab identified ALM-CS hyperconnectivity in *Sapap3* KO mice compared to wildtype (WT) controls using slice electrophysiology. We also observed increased ALM-CS activity in KOs vs. WTs during grooming by measuring bulk calcium signal with fiber photometry. To assess the functional relevance of this circuit during grooming, we carried out *in vivo* optogenetic inhibition of the ALM-CS circuit during spontaneous grooming bouts. We found reductions in groom bout length in *Sapap3* KO mice expressing ArchT in the ALM-CS circuit compared to eYFP-expressing controls, without a corresponding laser-induced decrease in locomotor activity or speed. This suggests a specific effect on grooming behavior rather than a general motor deficit (n = 8 WT/eYFP (4M/4F), 10 WT/ArchT (5M/5F), 7 KO/eYFP (3M/4F), 7 KO/ArchT (3M/4F)). To examine circuit dynamics during freely-moving behavior, we also performed *in vivo* single-photon calcium imaging (Inscopix) through implanted GRIN lenses in mice expressing GCaMP6m within the ALM-CS circuit. In WT mice, we found increased calcium activity in the ALM that peaked prior to the cessation of a grooming bout; analysis of KO calcium activity is ongoing and will be presented at the meeting. Together, these findings support a role for ALM-CS projections in maintaining compulsive-like behavior and highlight this circuit as a potential therapeutic target for disorders of compulsivity.

Disclosures: G.G. Liu: None. **S.E. Ahmari:** None.

Poster

PSTR077: Motor Planning and Execution: Behavior, Neurophysiology, and Imaging

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR077.26/P8

Topic: F.04. Voluntary Movements

Title: Electroencephalography power differences between reach and rest in young infants.

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Abstract: Background: Understanding how brain function relates to human movement in infancy is key to identifying motor control processes in early development. Research often focuses on the sensorimotor rhythm or “mu rhythm” due to its functional associations with

movement. Using electroencephalography (EEG), the mu rhythm is detected over sensorimotor cortex in the 6-9 Hz frequency band in infants. It is present at rest and attenuates with movement, making it a promising marker of motor control. Mu rhythm is typically expressed using power, reflecting the amount of electrical activity within a frequency range. Relative power, the proportion of power in a target frequency range relative to the total power, is typically reported as it accounts for individual differences in signal strength and improves comparability across infants. However, this functional association between mu rhythm and movement has not been reported in infants under 9 months of age. To address this gap, our study investigated EEG relative power differences (in the 6-9 Hz frequency) between reach and rest conditions in infants under 9 months old. We hypothesized relative power will be different between the baseline (i.e., rest) and reach conditions, reflecting attenuation of mu activity during movement. **Methods:** EEG data were recorded from 20 infants with typical development (mean age = 7.2 months, SD = 3 weeks) who were skilled reachers and could sit independently for at least 5 seconds. EEG was recorded while the infant sat on the caregiver's lap and repeatedly reached for a toy presented at midline at ~75% of arms reach. EEG preprocessing followed standardized, automated protocols and aligned with current best practices in developmental EEG research. The Fast Fourier Transform and Welch's method were used to compute Power Spectral Density, and from this, total and relative power in the 6-9 Hz were ascertained. Differences between reach and rest conditions were analyzed using a paired samples t-test. **Results:** Preliminary findings showed there was no significant difference in the 6-9 Hz frequency band between rest (M = 0.0015, SD = 0.0007) and reach condition (M = 0.0013, SD = 0.0006), $t(19) = 1.166$, ($p = .129$). **Conclusion:** Our preliminary results indicated no difference in means of relative power in mu between reach and rest. The small sample size may have limited statistical power, therefore increasing sample size in future analyses will be an important next step.

Disclosures: N.M. Remec: 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.; R01 HD101641-06 - Dr. Smith.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.01/P9

Topic: F.09. Motor Neurons and Muscle

Title: Development and characterization of humanized DMD mouse models for preclinical therapeutic evaluation

Authors: S. WANG, W. ZHONG, P. YOU, R. LV, ***J. ZHANG**, X. ZHOU;
Biocytogen, Waltham, MA

Abstract: Duchenne Muscular Dystrophy (DMD) is a severe X-linked genetic disorder caused by mutations in the *Dystrophin* gene, leading to progressive muscle degeneration and premature mortality. In this study, we utilized two distinct mouse models to investigate the pathophysiology of DMD and to develop a novel humanized model for therapeutic research.
We first generated a knockout mouse model, B-Dmd KO (del45-50) mice, with deletion of exons 45 to 50 in the *Dmd* gene. We confirmed the knock-out at both the mRNA and protein expression levels. To evaluate the functional impact of this genetic modification, we conducted grip strength and rotarod tests to assess muscle strength and motor balance, respectively. Results showed that B-Dmd KO (del45-50) mice exhibited significantly reduced forelimb grip strength and impaired motor balance compared to wild-type control mice, thereby effectively mimicking the motor deficits observed in DMD patients.
In recent years, exon-skipping oligonucleotide therapies have shown promise for DMD treatment. However, the *DMD* gene is the largest known human gene, making conventional gene editing approaches challenging. To address this, we developed a humanized knockout mouse model, B-hDMD (exon44-46, del45) mice, in which we replaced exons 44 to 46 in the mouse *Dmd* gene with human homologous sequences while deleting exon 45. This humanized knockout model was designed particularly for exon-skipping therapies targeting exon 44.
In the humanized knockout B-hDMD (exon44-46, del45) mice, we confirmed the presence of the human sequence in heart and skeletal muscles using RT-PCR, and western blot analysis demonstrated the loss of endogenous DMD protein following human sequence integration. Elevated creatine kinase (CK) activity, a biomarker of muscle damage in DMD, was detected in the serum of these humanized knockout mice. Additionally, histological examination via hematoxylin and eosin (H&E) staining revealed significant pathological changes in the muscles, including muscle cell atrophy, centralized nuclei, and inflammatory cell infiltration in the gastrocnemius and soleus muscles.
In conclusion, our study successfully developed two mouse models that recapitulate key pathological and functional features of DMD. The humanized knockout B-hDMD (exon44-46, del45) mouse model, in particular, provides a valuable platform for preclinical evaluation of exon-skipping therapies and other novel treatments targeting DMD. Future research will focus on further characterizing the disease progression in these models and exploring their potential applications in therapeutic development.

Disclosures: S. Wang: None. **W. Zhong:** None. **P. You:** None. **R. Lv:** None. **J. Zhang:** None. **X. Zhou:** None.

Poster

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Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.02/P10

Topic: F.09. Motor Neurons and Muscle

Title: Characterization of skeletal muscle cells derived from primary and immortalized myoblasts

Authors: C. VAJHØJ[1], A. LASSEN[1], J.-S. GRIGOLEIT[2], M. AMBJORN[3], ***T. C. STUMMANN**[1];
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Abstract: There is a significant need for therapies to treat neuromuscular diseases and other conditions associated with muscle weakness, such as Amyotrophic Lateral Sclerosis and the recovery of muscle function after surgeries involving neuromuscular blocking agents. Developing these therapies requires suitable cell-based assays to identify and select compounds for further development. Commercially available primary human and immortalized skeletal muscle precursors (myoblasts) have potential to be used for this purpose. Primary cells allow the study of genetically diverse donors but have limited expansion capacity and batch/donor variation, which can hinder robust assay development. In contrast, cell lines can expand indefinitely, making them suitable for screening assays and genetic modifications, but they lack genetic diversity and derivate from their origin due to the immortalization process. Regardless of their source, myoblasts can differentiate into multinucleated myotubes (young skeletal muscle fibers) that express skeletal muscle markers. However, there are limited reports on their ability to respond to neuronal signalling, which is crucial for establishing neuromuscular junction models. We characterized myotubes differentiated from immortalized mouse C2C12 myoblasts, immortalized human LHCN-M2 myoblasts, and human primary myoblasts and examined the expression of skeletal muscle maturity markers using immunocytochemistry. Intracellular cytosolic calcium or plasma membrane potential-sensitive probes and fluorescence kinetic imaging was used to assess the functional response to either acetylcholine (ACh, the main neurotransmitter of the neuromuscular junction) or potassium chloride (KCl, to depolarize their plasma membrane) to mimic neuromuscular junction neuronal input. Myosin heavy chain expression was confirmed in all three cultures, with the largest population of positive cells in the myotubes differentiated from primary myoblasts. Myotubes from all sources responded in a concentration-dependent manner to ACh and potassium chloride, but the three cultures showed differences in response amplitudes and well-to-well variation. Overall, our results support the use of *in vitro* derived myotubes for establishment of simplified neuromuscular junction models.

Disclosures: C. Vajhøj: A. Employment/Salary (full or part-time): H. Lundbeck A/S. **A. Lassen:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **J. Grigoleit:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **M. Ambjorn:** A. Employment/Salary (full or part-time): H. Lundbeck A/S. **T.C. Stummann:** A. Employment/Salary (full or part-time): H. Lundbeck.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.03/P11

Topic: F.09. Motor Neurons and Muscle

Title: Inactivation of MITF by histone deacetylase 8 represses myogenic differentiation via miR-18b/CTGF/TrkA/Erk1,2 signaling pathway in Duchenne muscular dystrophy

Authors: *K. CHOI;
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Abstract: Duchenne muscular dystrophy (DMD) is a degenerative disorder caused by the lack of dystrophin. Dystrophin is connected to the transmembrane complex called a dystroglycan complex, which maintains the myotube structure and its integrity. The absence of dystrophin leads to muscle degeneration without regeneration due to repeated sheer force of skeletal muscle. HDAC8 is a histone deacetylase enzyme that is significantly increased in DMD. Studies have found that HDAC8 inhibits myogenic differentiation of skeletal muscle. Here, we studied how HDAC8 changes the molecular mechanism of myogenesis. Microphthalmia-associated transcription factor (MITF) mediates the formation of myotube. Connective tissue growth factor (CTGF/CCN-2) is involved in the induction of fibrosis in muscle degenerative diseases. In *mdx* mouse, a DMD mouse model, CTGF is significantly increased whereas the dystrophin is decreased. Taken all together, our data indicate that myogenesis is affected by epigenetic modification changing the intracellular signaling pathway. Overexpressed HDAC8 in differentiated myoblast represses MITF. miR-18b is involved in the regulation of CTGF and mediated by MITF. miR-18b modulated by MITF at its promoter region plays a significant role in the regulation of CTGF expression, which would not only promote the development of fibrosis but also inhibit the myogenesis. Our study suggests a novel mechanism to understand the pathophysiology of DMD.

Disclosures: K. Choi: None.

Poster

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Program #/Poster #: PSTR078.04/P12

Topic: F.09. Motor Neurons and Muscle

Support: R01NS118066

Title: Deciphering the Role of ATL1 mutant astrocytes in SPG3A pathogenesis via NF-κB pathway using patient-specific stem cells

Authors: *G. THAKUR[1], A. KHADILKAR[2], L. KONG[4], S.-C. ZHANG[5], X.-J. LI[3];
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Abstract: Hereditary Spastic Paraplegia type 3 A (SPG3A) is an autosomal dominant pure neurodegenerative disorder resulting from mutations in the ATL1 gene that leads to axonal

degeneration and neurological dysfunction. Despite established neuronal involvement, the role of astrocytes, critical regulators of brain homeostasis and inflammation, in SPG3A pathology is largely unknown. This study investigates the role of mutant ATL1 on the human astrocyte-driven neuroinflammation and neurodegeneration, which is associated with co-activation of the NF-κB signaling pathway. Human pluripotent stem cells (hPSCs) that are knocked-in ATL1 mutations (ATL1-161 and ATL1-239) and healthy controls (H9) were differentiated into cortical astroglial cells by a multi-stage in-vitro protocol involving embryonic bodies, neuroepithelial aggregates, and glial progenitors. The astroglial cells were identified by immunofluorescence for S100B and GFAP. The anti-inflammatory effect of LXR agonists on the secretion of pro-inflammatory factors, IL-6 and TNF-α induced by polyinosinic polycytidylic acid (PIC). Western blot analysis was performed to evaluate the expression and phosphorylation of key NF-κB pathway components, including NF-κB p65 and NF-κB2 p100/p52. Our findings demonstrate that ATL1 mutations in human astrocytes lead to increased neuroinflammation. Specifically, PIC treatment significantly elevated IL-6 and TNF-α secretion in ATL1-mutated astrocytes, along with a marked upregulation of TLR mRNA levels in mutant astrocytes. Importantly, the levels of IL-6 and TNF-α in the ATL1-mutated astrocytes were significantly reduced following treatment with LXR agonist, suggesting an anti-inflammatory effect. Moreover, preliminary western blot data indicate alterations of NF-κB pathway in ATL1-mutated astrocytes compared to controls. These data strongly support a role for ATL1 mutations in human astrocytes in neuroinflammation, probably by disrupting the NF-κB pathway. The ability of LXR agonist to ameliorate inflammation in ATL1-mutant astrocytes presents a potential therapeutic strategy to reduce neuroinflammation and possibly neurodegeneration in SPG3A. Additionally, complementary transcriptomic and lipidomic studies, as well as assessment of ER morphogenesis are ongoing to fully understand the intricate molecular mechanisms of astrocyte pathogenesis in SPG3A.

Disclosures: G. Thakur: None. **A. Khadilkar:** None. **L. Kong:** None. **S. Zhang:** None. **X. Li:** None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.05/Q1

Topic: F.09. Motor Neurons and Muscle

Title: Assessment of monoethlycholine as a post-exposure therapy for nerve agents in guinea-pigs

Authors: *J. GUNN, **C. WHITMORE**;
Defence Sci. and Technol. Lab., Salisbury, United Kingdom

Abstract: Nerve agents (NAs) inhibit acetylcholinesterase (AChE), resulting in an accumulation of acetylcholine (ACh) at cholinergic synapses. The resulting overstimulation of both muscarinic

(mAChR) and nicotinic (nAChR) receptors leads to the toxic effects of NAs which include muscular paralysis, seizures and respiratory depression. Current treatments use a muscarinic antagonist to reduce the overstimulation at mAChRs, an oxime to restore AChE activity and a benzodiazepine to treat seizures. The oxime is the only component targeting overstimulation of the nAChR. This has drawbacks because no single oxime has adequate reactivating activity against all NAs [1]. Reducing the overstimulation of the nAChR without reactivation of inhibited AChE could provide a therapeutic benefit for any NA. Here, monoethylcholine (MECh) has been assessed, in vivo, for its ability to provide improved survival benefit following NA exposure by reducing nAChR activity. MECh is taken up into cholinergic nerve terminals and substitutes for choline, making the false transmitter acetylmonoethylcholine (AMECh). AMECh is less efficacious at the nAChR than ACh and MECh has some direct antinicotinic but little antimuscarinic effect [2]. These effects restore function in NA-exposed hemidiaphragm preparations in a manner consistent with production of AMECh [2]. *G*uinea pigs were challenged s.c. with 80 µg/Kg (~2.2 × LD_{50}) soman and one minute later received either atropine and midazolam or atropine, midazolam and MECh i.m. All 8 animals receiving atropine and midazolam died between 15 and 270 min, whereas a statistically significant survival benefit was seen with addition of MECh; 7 of 8 animals survived to the end of the study at 6 h. Animals receiving MECh displayed signs of NA exposure, including a hypothermia which improved by the end of the study. A preliminary analysis of neuromuscular function in hemidiaphragms from animals treated with MECh identified a reduction in the fade of the 50 Hz tetanus when compared to animals receiving the control therapy. This is consistent with therapeutic benefit due to production of the false transmitter AMECh. References: [1] Tattersall JEH. *(2018) Curr. Opinion Physiol.*, 4:49-56*;* [2] Whitmore et al*.,* (2020) *Tox. Letters,* 321: 21-31 © Crown copyright (2025), Dstl. This information is licensed under the Open Government Licence v3.0. To view this licence, visit https://www.nationalarchives.gov.uk/doc/open-government-licence/. Any enquiries regarding this publication should be sent to: centralenquiries@dstl.gov.uk.

Disclosures: J. Gunn: A. Employment/Salary (full or part-time): Defence Science and Technology Laboratory. **C. Whitmore:** A. Employment/Salary (full or part-time): Defence Science and Technology Laboratory.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.06/Q2

Topic: F.09. Motor Neurons and Muscle

Support: University of Costa Rica (742-B4-318)
Fondazione AIRC per la Ricerca sul Cancro (grant #IG 21558)
Italian Research Ministry (PRIN 20174TB8KW)
Fondazione Telethon (grant #GMR22T102)
Fondazione Telethon/Cariplo (grant #GJC22008)

Title: Structural differences between the human ClC-1 channel and the classical ClC topology commonly used: an update in domains and functions.

Authors: *O. BRENES[1,2], F. MORALES[3,4], M. PUSCH[5];
[1]Dept. of Physiol., Univ. of Costa Rica, Montes de Oca, Costa Rica; [2]Ctr. of Neurosci. Res., [4]Inst. de Investigaciones en Salud, [3]Univ. of Costa Rica, San José, Costa Rica; [5]Inst. di Biofisica, Genova, Italy

Abstract: The chloride voltage-gated channel 1 (ClC-1) expressed in skeletal muscle cells has been extensively studied since mutations in the gene that encodes this channel are responsible for a common disease called Myotonia Congenita. Loss-of-function of the ClC-1 channel evokes a hyperexcitable state in muscle cells characterized by sustained bursts of action potentials in the muscle fibers, leading to involuntary aftercontractions, muscle stiffness, and hypertrophy. The ClC chloride channel structure was first described in a seminal work by Dutzler et al (2002). Using *Salmonella enterica* and *Escherichia coli* chloride channels X-ray structures, Dutzler showed the presence of a homodimeric channel, and each subunit contained 18 α-helices (named from A to R), where the C-terminal half ran in an antiparallel arrangement with the N-terminal half. This topological structure has been extensively used in the literature to localize mutations in the ClC-1 muscle channel, related to Myotonia Congenita, thereby enhancing our understanding of this disease. In 2018, Park and MacKinnon published the 3D structure of the human ClC-1 channel. However, the topology of the human channel was not provided in this study. Our extensive analysis of the human ClC-1 structure revealed some subtle yet essential differences in the channel topology compared to the classical topology typically used. The most significant differences are the absence of loops and the presence of more than 18 α-helices, some of which expand in an intracellular or extracellular fashion. In this poster, we present the differences in the topology of the classical chloride channel and the human ClC-1 channel. In addition, in order to keep the original Dutzler classification of α-helices, we propose an additional nomenclature for the new helical segments. Moreover, we present the precise amino acid residues predicted for the beginning and end of each α-helix, as well as the extracellular, intracellular, and intramembrane amino acids, providing the scientific community studying myotonic mutations with the opportunity to properly localize ClC-1 mutations in humans. Finally, we performed an analysis of the functional effects described for naturally occurring mutations in the α-helices of the human channel, allowing an update in the known functions of each domain.

Disclosures: O. Brenes: None. **F. Morales:** None. **M. Pusch:** None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.07/Q3

Topic: F.09. Motor Neurons and Muscle

Title: Coupling Compartmentalized Microfluidic Platforms with MEA for Advancing Neuromuscular Junction Modeling

Authors: N. BAGDADI, *T. HONEGGER;
NETRI, Lyon, France

Abstract: Neuromuscular disorders pose significant challenges for therapeutic intervention, primarily due to the lack of accessible predictive models for studying the neuromuscular junction (NMJ). Improving the predictive accuracy of these models offers tremendous potential for uncovering the mechanisms governing NMJ development, maintenance, and pathology. Induced pluripotent stem cells (iPSCs) enable the creation of human neuromuscular junction (NMJ) models for studying synaptic development, maturation, and disease mechanisms while advancing drug discovery. Combining iPSCs with NETRI's advanced technologies is crucial for developing robust models that drive therapeutic innovation. The complex interactions among motoneurons and skeletal muscle cells require precise *in vitro* NMJ models, essential for studying NMJ-related pathologies like antibody-mediated damage, ion channel dysregulation, and disorders such as myasthenia gravis, Lambert-Eaton syndrome, and acquired neuromyotonia. These models could also provide insights into conditions like ALS, SMA, and muscular dystrophies, supporting the development of targeted therapies. Our pioneering work combined NETRI's Dualink MEA microfluidic technology with hiPSC-derived motor neurons (MNs) and human skeletal muscle derived primary myoblasts. Compartmentalized and microfluidically isolated devices offer the advantage of modular assessment of the joint muscular and motoneuron models. Individual electrophysiology recordings consistent with stimulation on neuronal compartment are correlated with markers for myotube activity modulation in the muscular compartment. Also, the specific architecture of NETRI's devices including specific readouts (immunofluorescence and MEA recording) allowed the assessment of skeletal muscle equivalent response to motoneuron pharmacological or electrical stimulation. This integration highlights the potential of NETRI's platforms to enhance the accuracy and translational relevance of generated human NMJ models, driving advancements in therapeutic discovery and development.

Disclosures: N. Bagdadi: None. **T. Honegger:** None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.08/Q4

Topic: F.09. Motor Neurons and Muscle

Title: Automation of Toxin Testing Using a High-Throughput Human Neuromuscular Junction-on-a-Chip Platform

Authors: *M. MAGDESIAN[1], R. DA SILVA[2], L. KUYLTJES[1], A. IZADI[1];
[1]Ananda Devices, Laval, QC, Canada; [2]Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Background and Purpose: Neuromuscular junctions (NMJs) are critical for signal transmission between motor neurons and skeletal muscles and are highly vulnerable to toxins. Current NMJ toxicity models lack human relevance, scalability, and rely heavily on animal testing, limiting accurate neurotoxicity prediction. To overcome these limitations, Ananda Devices developed NeuroMuscle™, a human-based micro physiological platform that replicates functional NMJs in vitro. Validated for high-throughput toxicity testing and drug discovery, the platform enables real-time monitoring of NMJ integrity in response to neurotoxins. Botulinum toxin (BoNT), due to its potency and medical relevance, is used to demonstrate the platform's capabilities. NeuroMuscle™ presents a human-relevant, animal-free solution for studying NMJ-targeted toxicity and developing medical countermeasures (MCMs). **Methods:** NeuroMuscle™ assembles iPSC-derived human motor neurons and skeletal muscle cells into functional NMJs within a microfluidic device. It supports real-time monitoring of NMJ, neuronal, and muscle activity via brightfield live imaging using standard microscopes. Integrated software enables high-sensitivity analysis of cellular parameters in under one minute. Unlike PDMS-based systems, the platform is compatible with standard microscopy setups and offers reproducibility across batches. Its automated, high-throughput design supports rapid, sensitive testing, suitable for pharmaceutical development and toxin detection. **Results:** NeuroMuscle™ has shown robust detection of multiple BoNT subtypes at picogram sensitivity and faster timelines (within hours), surpassing the traditional mouse lethality assay. It delivers high-resolution, real-time data on NMJ function and integrity using minimal reagents (<50 μL) and detects toxins in human fluids. Its scalability has supported the development and approval of two new neuromuscular therapies. The ability to monitor both acute and recovery phases of intoxication provides comprehensive insights into toxin action and countermeasure efficacy. **Conclusions:** NeuroMuscle™ advances neurotoxicity testing by offering a scalable, reproducible, high-throughput alternative to animal models. It empowers researchers to rapidly evaluate BoNT and related neurotoxins, accelerating drug discovery and MCM development with enhanced precision and reduced animal use.

Disclosures: M. Magdesian: None. **R. Da Silva:** None. **L. Kuyltjes:** None. **A. Izadi:** None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.09/Q5

Topic: F.09. Motor Neurons and Muscle

Title: Hindbrain circuits for the production of different mouse vocalization types

Authors: *P. ZIOBRO, D. ZHENG, K. A. TSCHIDA;
Dept. of Psychology, Cornell Univ., Ithaca, NY

Abstract: The nucleus retroambiguus (RAm) is a hindbrain premotor region that receives input from the periaqueductal gray (PAG) and projects to downstream motor centers involved in vocal production. Previous studies have found that RAm regulates the production of two vocalization

types in mice: ultrasonic vocalizations (USVs) and distress calls (squeaks). Whether distinct vs. overlapping populations of RAm neurons regulate the production of these two vocalization types remains unclear. In the present study, we employed activity-dependent labeling methods to investigate the spatial distribution of RAm neurons that upregulate Fos expression in association with each vocalization type. Preliminary results suggest limited overlap between RAm neurons that upregulate Fos expression in association with USVs vs. squeaks and support a model in which some RAm neurons are active during both vocalization types, while others are only active during one vocalization type. These experiments lay a foundation for future work to test the functional contributions of these different subsets of RAm neurons to vocalization.

Disclosures: P. Ziobro: None. **D. Zheng:** None. **K.A. Tschida:** None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.10/Q6

Topic: F.09. Motor Neurons and Muscle

Support: the STI 2030-Major Projects, 2022ZD0208605, 2022ZD0208600, 2024YFC2510800, 2022ZD0210000
the National Natural Science Foundation of China, 82072545, 82021002, T2325008
Biomedical Technology Support Projects of Shanghai Science and Technology Innovation Action Plan, China, 22S31902100

Title: A wireless and fully implantable optogenetic stimulation system for long-term function evaluation of mice forelimb with sub-nerve resolution

Authors: *S. JIANG;
1.Department of Hand Surgery, Huashan Hospital, Fudan Univ., Shanghai, China

Abstract: Motor function defect caused by central or peripheral nerve injuries has become a global burden. Peripheral nerve transfer is an effective surgical method in restoring motor functions of upper limb after nerve injuries in general. However, the outcome of individual function recovery is less predictable. It is crucial to access the long-term evaluation of function improvement in the peripheral nerve level. Here, we developed a fully implantable multisite optogenetic stimulation system (FIMOSS), which is tailored for wireless, reprogrammable, and long-term function evaluation of peripheral nerve plexus. In Thy1-ChR2-EYFP mice, our system induced distinct compound muscle action potentials and forelimb movements when illuminating different nerve fascicles from different angles, confirming its spatial selectivity for the precise function evaluation of brachial plexus. Furthermore, we applied FIMOSS on a nerve transfer mice model after traumatic brain injury and discovered innervation pattern of the transferred nerve to multiple forelimb muscles along with the functional compensation of adjacent nerve

trunks in brachial plexus consecutively within 12 weeks after surgery, during which FIMOSS has demonstrated stable operation. Our technology enabled refined evaluation of electrophysiological and motor functions of peripheral nerve plexus, shining light upon personalized diagnosis and treatment after central or peripheral nervous system injuries or nerve surgeries.

Disclosures: S. Jiang: None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.11/Q7

Topic: F.09. Motor Neurons and Muscle

Title: C1q Inhibition preserves the structure and function of neuromuscular junctions in a rat model of Myasthenia gravis(MG)

Authors: *J. VEREEN;
Annexon Biosci., Brisbane, CA

Abstract: Myasthenia gravis (MG) is a chronic autoimmune disorder characterized by the disruption of synaptic connectivity at the neuromuscular junction (NMJ) due to autoantibody-mediated attack on postsynaptic components, often against acetylcholine receptors (AChR). In MG, it is well established that terminal complement activation, particularly the membrane attack complex formation, plays a crucial role in damaging NMJ and contributing to muscle weakness. However, the role of classical complement activation to the pathophysiology of the disease is less understood. Given autoantibodies found in most cases of MG are complement-fixing, we explored the possible involvement of C1q, the initiating molecule of the classical complement cascade, and upstream classical pathway activation in the pathophysiology of MG using a preclinical rat model. Here we show that C1q binds to autoantibody tagged AChRs at the NMJ, triggering classical pathway activation characterized by C4 deposition, ultimately resulting in loss of structural integrity of NMJs and corresponding muscle weakness. Inhibition of C1q using the mouse monoclonal antibody, ANX-M1.2, during the presymptomatic phase of the disease results in reduction of C1q levels in plasma and muscle tissue, prevents C4 deposition on NMJs, preserves NMJ integrity, and ameliorates muscle weakness. These results indicate that blocking initiation of the classical complement pathway via C1q inhibition may offer therapeutic benefit in MG by preserving NMJ structure and function.

Disclosures: J. Vereen: A. Employment/Salary (full or part-time): employment with and equity ownership in Annexon Biosciences.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.12/Q8

Topic: F.09. Motor Neurons and Muscle

Support: Education and Research of the Federal Republic of Germany (BMBF) by funding the project AI.D under the Project Number 16ME0539K

Title: Motor unit-based cursor control for tetraplegic individual with cervical SCI: Feasibility and limb-specific differences in myoelectric activity

Authors: *I. XYGONAKIS[1], A. R. CRAIK[1], M. ZAVAGLIA[1], S. HADDADIN[2];
[1]Tech. Univ. of Munich, Munich, Germany; [2]Mohamed bin Zayed Univ. of Artificial Intelligence (MBZUAI), Abu Dhabi, United Arab Emirates

Abstract: Cervical spinal cord injury (SCI) often impairs arm and hand function, limiting patients' ability to interact with their environment. In recent years, researchers have explored residual myoelectric activity in paralyzed hand muscles using high-density surface electromyography (HDsEMG). This approach has enabled offline decoding of multiple wrist and finger movements and real-time control of virtual hands, avatars, and hand orthoses in individuals with motor (in)complete SCI. In this study, we further evaluate HDsEMG as a human-computer interface in severe cervical SCI, focusing on: (1) the impact of paralyzed side (left vs. right) on decoding performance, and (2) the feasibility of motor unit-based cursor control.
A 45-year old male SCI subject (C5 complete), followed on-screen cues to perform sustained wrist movements. Two 64-channel HDsEMG grids were placed over the forearm on the flexor and extensor sides. Movements set: wrist flexion/extension, ulnar/radial deviation, pronation/supination, open hand, power and pinch grasp (trial duration: 18s, rest: 5s, 3 repetitions). Each side recorded in separate sessions.
For the movement decoding, first 2 trials were used for training, and the last one for testing. In the training set motor unit (MU) separation filters and spike thresholds were estimated. EMG decomposition was used, based on fastICA, and acceptance criteria (Silhouette coef.=0.7, CoV_ISI =2, duplicate synchronicity percentage 30%). Feature vectors were based on MU spike counts within 200 ms sliding windows, were classified using a support vector machine (SVM).
Initially the side analysis, showed significant difference between two extremities, quantified the in the MU yield (4/task) vs (10/task) and task-related RMS compared to rest. Regarding the movement decoding, only left side could be used, leading to 60% accuracy, while when the 4-best classes were used, enough for 2D control, accuracy increased to 85%.
Our results, on single subject, indicate that paralyzed side can have severe impact in the myoelectric activity, hence although paralyzed one side seems to have more residual MUs. Additionally, the MU-based classifier makes the real-time 1D cursor control feasible, something we plan to explore next.

Disclosures: I. Xygonakis: None. **A.R. Craik:** None. **M. Zavaglia:** None. **S. Haddadin:** None.

Poster

PSTR078: Motorneuron-Muscle Interface and Muscle Physiology/Biochemistry

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR078.13/Q9

Topic: F.09. Motor Neurons and Muscle

Support: R01 NS132728

Title: Modulating Motoneuron Activity to Influence Neuromuscular Junction Maturation in a Model of Cerebral Palsy

Authors: *E. GONZALEZ[1], E. REEDICH[2], K. A. QUINLAN[3], M. MANUEL[2];
[1]The Univ. of Rhode Island, South Kingston, RI; [3]Biomed. and Pharmaceut. Sci., [2]Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral palsy (CP) is a neurodevelopmental disorder that is often associated with hypoxia-ischemia (HI) injury to the developing brain, leading to motor impairments and lifelong disability. When HI injury occurs during a critical period of development in late gestation or early childhood, it can disrupt normal neuromuscular development. Neuromuscular junction maturation is highly activity-dependent, relying on precise patterns of motoneuron activity to shape synaptic structure and function. We hypothesize that HI-induced alterations in motoneuron activity disrupt neuromuscular junction development, leading to a delay in the transition between poly- to mono-innervation, and an immature morphology when mono-innervation is finally achieved. To test this hypothesis, we use a rabbit model of CP, which closely mimics the motor deficits and neurophysiological alterations seen in human CP. We will employ Adeno-associated-virus (AAVs) based gene delivery in neonatal HI rabbits and sham controls to selectively modulate motoneuron activity using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). This approach allows us to precisely control motoneuron excitability and examine its effects on neuromuscular junction structure and function. By comparing neuromuscular junction morphology and innervation pattern using immunofluorescence in normal and CP-affected animals with and without DREADD-induced activity modulation, we aim to determine the extent to which altered motoneuron activity drives neuromuscular junction dysfunction in CP. In initial experiments, we injected AAVs into the lumbricals and Levator Auris Longus (LAL) at post-natal day 1 (P1). These small muscles of the paw and ear, respectively, were selected due to their small size for whole-mount analysis. Our findings show successful transduction of a subset of motoneurons as early as postnatal day 5 (P5), with sustained expression at P8 and P11. These findings provide early validation of our gene delivery approach. Based on these results we will increase the activity of a subset of motoneurons between P4 and P11 using retroAAV-hSyn-hM3D(Gq)-mCherry and daily injections of the specific DREADD agonist JHU37160. Our study will offer critical insights into the role of motoneuron activity in neuromuscular junction development and offer potential therapeutic strategies for improving neuromuscular function in CP.

Disclosures: E. Gonzalez: None. **E. Reedich:** None. **K.A. Quinlan:** None. **M. Manuel:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.01/Q10

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Pregnant mice have increased co-localization of oxytocin receptor and GABA in the nucleus accumbens shell

Authors: *A. HAUPT[1], H. K. CALDWELL[2];
[1]Biol. Sci., Kent State Univ., Kent, OH; [2]Dept. of Biol. Sci., Kent State Univ. - Biol. Sci., Kent, OH

Abstract: The neuropeptide oxytocin (Oxt) is a known modulator of maternal behaviors and in rodents is important for the initiation of maternal care. During the peripartum period there are increases in the release of Oxt both peripherally and centrally. Immediately prior to and during parturition there is a rapid upregulation of the Oxt receptor (Oxtr) in numerous brain regions followed by a downregulation in the post-partum period. Oxtr expression has been found to be the highest in brain regions associated with the maternal behavior neural network (MBNN). The MBNN is made up of several brain areas where sensory cues, cortical inputs, hormones, and neurochemicals all coordinate to modulate the expression of maternal care. Two of these regions, the medial preoptic area and bed nucleus of the stria terminalis, integrate pup sensory cues and relay the information to the ventral tegmental area (VTA). The VTA then dampens nucleus accumbens (NAcc) output which then disinhibits the ventral pallidum (VP) allowing for the triggering of maternal responsiveness. Investigation into the role of Oxtr in the NAcc by our lab has found that disruption of Oxtr in the NAcc shell results in a complete failure in the initiation of maternal care. Given that Oxtr in the NAcc shell are Gq-coupled and therefore increase neuronal excitability, we speculated their co-localization with GABAergic neurons may enhance local inhibition within the NAcc shell - likely via interneurons - thereby dampening NAcc output to the VP and facilitating maternal responsiveness. Thus, we hypothesized there would be an increase in the co-localization of Oxtr and GABA in the NAcc shell of pregnant mice. To test our hypothesis, we utilized timed pregnancies of virgin C57BL/6 mice in order to collect brains at gestational day 19. We then used RNAscope to compare Oxtr and SLC32A1 (a GABA transporter marker) co-localization in the NAcc shell of pregnant mice and virgins to explore neurochemical changes that may underlie VP disinhibition. We found that the number of cells where Oxtr and SLC32A1 were co-localized was increased in the NAcc shell of pregnant females. We also found there were increases in the number of cells containing GABA in pregnant mice. We speculate that the increased co-localization is essential for the triggering of maternal responsiveness by reducing the inhibitory output of the NAcc, i.e., removing the 'break' on the VP. To our knowledge, these data are the first to show an increase in the co-localization of Oxtr and GABA in late-term pregnancy of mice. These findings advance our understanding of the neural circuitry underlying the onset of maternal behavior.

Disclosures: A. Haupt: None. **H.K. Caldwell:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.02/Q11

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NTU (NTU-CC-114L891304)
The National Science and Technology Council (NSTC 112-2311-B-002-007-MY3)

Title: The regulatory mechanism underlying oxytocin's central and peripheral release by Synapsin1a

Authors: *C.-T. HUANG, C.-T. WANG;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Oxytocin (OT) laden in large dense-core vesicles (LDCVs) of the hypothalamus neurons can be released either to central brain targets (i.e., central release) or to peripheral circulation via pituitary nerve terminals (i.e., peripheral release). Recent studies demonstrated that OT can regulate food intake and glucose homeostasis via central or peripheral effects; however, the regulatory mechanism mediating OT central and peripheral release remains unclear. A synaptic vesicle (SV)-associated protein Synapsin1a (Syn1a) is shown to regulate the LDCV release in a phosphorylation-dependent manner, leading to a hypothesis that Syn1a may regulate OT release from LDCVs. Here, we aimed to investigate how Syn1a regulates the OT central and peripheral release. We designed a series of constructs driven by the OT neuron-specific promoter to overexpress a OT carrier protein Neurophysin1 (Np1) fused with pHVenus, with optional overexpression of Syn1a or its phosphodeficient mutants. We performed *in vivo* electroporation to transfect the rat hypothalamus (supraoptic nucleus; SON) OT neurons with these constructs, including intact control (IC), sham control (SC), Syn1a, and the Syn1a phosphodeficient mutant on the MAP kinase site (designated MAPK*) or CaM kinase sites (designated CaMK*). We measured the OT levels in the cerebrospinal fluid (CSF) and plasma before and after a week of transfection. We compared the changes in OT levels after transfection in the same rats for each group. As a result, only SC showed a significant increase in the CSF OT level after transfection, suggesting that overexpressing Np1 may enhance OT central release. In contrast, plasma OT levels were significantly decreased after transfection in the Syn1a-MAPK* and CaMK* groups. By comparing the effects among different groups, the CSF OT level was significantly increased in SC compared to other groups after transfection. The increase in CSF OT level was significantly occluded by overexpressing Syn1a in the SON neurons compared to SC, suggesting that overexpressing Syn1a dampens the Np1-enhanced OT central release. On the other hand, the plasma OT level was significantly decreased by overexpressing Syn1a-MAPK* compared to Syn1a, suggesting that a deficiency in MAPK-mediated Syn1a phosphorylation

inhibits OT peripheral release. Thus, Syn1a overexpression tends to reduce the OT central release to CSF, and a deficiency in MAPK-mediated Syn1a phosphorylation further inhibits the OT peripheral release. Our findings open a new avenue in the study of OT release, highlighting the importance of regulation through post-translational modification of the release machinery.

Disclosures: C. Huang: None. **C. Wang:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.03/Q12

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: Middlebury College SRPS

Title: Effects of testosterone upon the survival of new neurons in the dentate gyrus of adult male rats

Authors: *M. D. SPRITZER[1], T. W. BRAUN[2], K. A. BLEK[3], J. M. GARDNER[2], E. J. LEMOLE[2], G. K. NADECKI[3];
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Abstract: Adult neurogenesis has been implicated in mood and memory disorders, and substantial work has established that testosterone enhances hippocampal neurogenesis by increasing the survival and not the proliferation of new neurons. Recent research from our laboratory has shown that the sex steroid hormone testosterone has neuroprotective effects upon the later stages of neural development in the dentate gyrus. This experiment aimed to further elucidate during which stages of cellular development testosterone has its neurogenesis-enhancing effects, with a focus on the later stages of neural development (15-30 days after cell proliferation) when functional synapses are forming. Adult male Sprague-Dawley rats (n = 7-8/group) were bilaterally castrated and given a single injection of bromodeoxyuridine (BrdU; 200 mg/kg) on the first day of the experiment to label actively dividing cells. Rats were then injected daily with testosterone (0.500 mg/rat) or sesame oil (0.1 ml/rat) from days 1-30, 1-15, or 16-30 after BrdU injection. Transcardial perfusions and brain extractions were then conducted, and tissue was sectioned (40 μm) through the rostral-caudal extent of the hippocampus. Peroxidase immunohistochemistry was used to visualize BrdU-labeled cells. Light microscopy (100x lens) was used to count all labeled cells in every 10th section throughout the dentate gyrus, with counts separated based upon cell layer (granule cell layer vs. hilus) and region (dorsal vs. ventral). Fluorescent double-labeling of tissue (BrdU and NeuN) followed by confocal microscopy was used to determine if any of the treatments influenced the probability that newly proliferated cells developed into neurons. We observed that testosterone-injected rats had more BrdU-labeled cells than the oil control group overall, and the effect was strongest in rats that

were injected during days 1-30 and 16-30. Confocal microscopy confirmed that a majority (about 80%) of BrdU-labeled cells had developed into neurons. These results support past findings in demonstrating that testosterone influences adult neurogenesis by enhancing the survival of cells in the later stages of maturation into neurons. It remains unclear whether this neuroprotectant effect of testosterone is unique to newly generated neurons or is a broader effect upon all neurons in the hippocampus. Given the potential benefits of adult neurogenesis for treating neuro-degenerative conditions (e.g., chronic depression and dementia), our results suggest that androgen therapies may be a useful treatment for male patients.

Disclosures: M.D. Spritzer: None. **T.W. Braun:** None. **K.A. Blek:** None. **J.M. Gardner:** None. **E.J. LeMole:** None. **G.K. Nadecki:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.04/Q13

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01 MH121603
NIH Grant R03 MH120549
GSU Brains and Behavior Seed Grant Program

Title: Chemical anatomy and connectivity of vasopressin 1a receptor in the lateral septum

Authors: *D. R. HARTSWICK[1], N. SCHAPPAUGH[1], G. J. DE VRIES[2], A. PETRULIS[1]; [1]Neurosci. Inst., [2]Dept. of Biol., Georgia State Univ., Atlanta, GA

Abstract: The neuropeptide arginine-vasopressin (AVP) regulates sexually differentiated social behaviors, such as sexual behavior, aggression, and social communication. Much of AVP's effect on social behavior is mediated by the most widely expressed AVP receptor in the central nervous system: the vasopressin 1a receptor (V1aR). V1aR is densely expressed in males and females in the lateral septum (LS), which receives heavy input from sexually differentiated populations of AVP cells in the bed nucleus of the stria terminalis (BNST) and the medial amygdala (MeA). Utilizing RNAscope in situ hybridization, we measured V1aR mRNA expression and colocalization of V1aR and oxytocin receptor (OTR), which also has a high affinity for AVP, in subregions of the LS. We found similar percentages of cells containing V1aR+ puncta across dorsal (LSd) and intermediate (LSi) LS in males (LSd: 70%, LSi: 71%) and females (LSd: 68%, LSi: 68%). The ventral LS (LSv) contained a smaller percentage of V1aR+ cells in both males (46%) and females (50%). A higher percentage of cells coexpressed V1aR and OTR in the LSi (male: 51%; female: 41%) than in the LSd (male: 38%; female: 31%) or LSv (male: 33%; female: 31%). To identify connectivity of V1aR LS cells, we used a monosynaptic retrograde rabies virus as well as anterograde synaptophysin-mRuby AAV tracing in our V1aR-Cre mouse line. Rabies tracing identified monosynaptic inputs to V1aR LS cells

from the lateral hypothalamus (LH), ventral hippocampus, medial septum, diagonal band of Broca (DBB), and the supramammillary nucleus (SuM). Synaptophysin labeling revealed outputs to some of these same structures (LH, DBB, SuM) as well as the lateral part of the anterior hypothalamic nucleus (lAH), medial/lateral habenula, medial preoptic area, and ventral pallidum. Synaptophysin-expressing viral injections in both the LSd and LSi show denser projections to lAH, LH, and habenula than those originating exclusively in the LSi. Characterizing the phenotype of LS V1aR cells and tracing their inputs and outputs in male and female mice will increase our understanding of how the AVP/V1aR system is organized and provide insight into how this circuit affects sexually differentiated social behavior.

Disclosures: D.R. Hartswick: None. **N. Schappaugh:** None. **G.J. De Vries:** None. **A. Petrulis:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.05/Q14

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01MH072930

Title: Neuroanatomical and functional characterization of lateral hypothalamic vasopressin 1a receptor-expressing cells in mice

Authors: *A. ZAW[1,2], N. SCHAPPAUGH[2], T. BUDEIR[2], G. J. DE VRIES[3], A. PETRULIS[4]; [2]Neurosci. Inst., [3]Dept. of Biol., [1]Georgia State Univ., Atlanta, GA; [4]Neurosci. Inst., Georgia State Univ. Neurosci. Inst., Atlanta, GA

Abstract: The vasopressin 1a receptor (V1aR) plays a key role in the neuropeptide vasopressin's (AVP) ability to regulate social behavior, arousal, and stress responses. Brain regions like the lateral septum (LS) and ventral pallidum show high V1aR mRNA expression and are well-studied in the context of social behavior. However, the organization and function of V1aR-expressing cells in the lateral hypothalamus (LH) remain poorly understood despite having both dense V1aR expression and strong AVP innervation. Using synaptophysin-based viral tracing from AVP-expressing neurons in the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus of the hypothalamus, we found that LH V1aR+ cells receive strong input from these vasopressinergic sources. The precise spatial distribution and downstream targets of these AVP inputs have been unclear, in part due to the diverse neuronal populations within the LH that express V1aR; here, we began to address this by characterizing their anatomical organization and projection patterns. RNAscope analysis revealed that these LH V1aR+ cells colocalized with several neuropeptidergic populations, including hypocretin/orexin (Hcrt) and melanin-concentrating hormone. Notably, most Hcrt+ neurons co-express V1aR mRNA, and females show greater Hcrt+ cell numbers and V1aR expression per cell than males. We observed

that these V1aR+ Hcrt+ cells are activated during social interactions in a sex-specific patterns: V1aR+ Hcrt+ neurons showed reduced Fos expression in males during male-female interactions, whereas the opposite pattern was observed during same-sex encounters. We also used an anterograde viral tracer strategy to study the outputs of these cells and observed that LH V1aR+ cells project to several brain regions, including the LS, horizontal and vertical limbs of the diagonal band (HDB and VDB), lateral habenula, and the supramammillary nucleus, with the intermediate LS and VDB emerging as prominent projection targets. While subtle sex differences in fiber density were observed, they did not reach statistical significance. We are mapping afferent inputs to these LH V1aR+ cells using monosynaptic rabies tracing and are knocking down V1aR in the LH using CRISPR-based tools to assess sex-specific behavioral changes. These studies provide critical insights into the neural circuitry and behavioral roles of sexually differentiated AVP signaling and their actions within the LH.

Disclosures: A. Zaw: None. **N. Schappaugh:** None. **T. Budeir:** None. **G.J. De Vries:** None. **A. Petrulis:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

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Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NRF Grant NRF-2022M3A9F3094559
NRF Grant RS-2023-00208193
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NRF and Ministry of Education Grant BK21 FOUR

Title: Brain-wide mapping of β-Klotho (KLB)-expressing neurons reveals discrete FGF21 target regions in mice

Authors: *H. KIM, Y. JEONG;
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Abstract: Fibroblast growth factor 21 (FGF21) is a metabolic hormone induced by nutritional challenges such as fasting, high-fat diet, low-protein diet, and high-sucrose intake. It is primarily produced in the liver and acts on β-Klotho (KLB)-expressing cells in target tissues including brown adipose tissue, pancreas, and the brain. FGF21 has been shown to influence alcohol intoxication and macronutrient preference, but the specific neural substrates through which it acts in the brain remain poorly defined. In particular, the spatial distribution and cellular identity of KLB-expressing neurons have not been systematically mapped in the brain, as previous studies have primarily used pharmacological or whole-body genetic approaches with limited spatial and cellular resolution. To address this, we developed an adeno-associated virus

expressing Cre recombinase under a 2 kb Klb promoter (AAV-Klb-Cre) and delivered it either systemically to postnatal day 0 (P0) Rosa26-tdTomato mice or stereotaxically into specific adult brain regions, such as the nucleus of the solitary tract (NTS). This dual approach enabled both brain-wide and region-specific genetic access to KLB+ neurons. We observed robust tdTomato labeling in circumventricular organs (e.g., area postrema, subfornical organ) and brainstem nuclei including the NTS, as well as in forebrain regions such as the hippocampal formation, retrosplenial cortex, and hypothalamic nuclei. These spatially restricted expression patterns suggest region-specific regulation of Klb promoter activity and heterogeneous access points for FGF21 signaling across central circuits. This system enables targeted manipulation of region-specific KLB+ neurons, allowing investigation into their roles in metabolic regulation, alcohol-induced arousal, and nausea-related behaviors.

Disclosures: H. Kim: None. **Y. Jeong:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.07/Q16

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIH R21 MH133228
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R01 DK136284
R01 DK131446
R01 DK135212

Title: Neural encoding of stress scalability by arcuate GABAergic neurons

Authors: Y. CAO[1], C. SU[2], F. H. DO MONTE[3], Q. TONG[4], *Y. XU[5];
[1]The Univ. of Texas MD Anderson Cancer Ctr. UTHealth Houston Grad. Sch. of Biomed. Sci., Houston, TX; [2]UTHealth at Houston, Houston, TX; [3]Neurobio. and Anat., Univ. of Texas at Houston, HOUSTON, TX; [4]IMM, Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX; [5]Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: An appropriate stress response is essential for proper responding to, coping with, and subsequently recover from disturbing environmental stressors. However, the dysregulated responses to stress stimuli can result in abnormal psychiatric and behavioral outcomes, and if severe, can lead to stress-related mental disorders such as the posttraumatic stress disorder (PTSD). To develop effective therapies for treating these disorders, it is of utmost importance to fully understand the brain neural bases and mechanisms regulating stress responses. Emerging evidence indicates that stress-related emotional modalities are equally implicated in feeding regulation by hypothalamic neurons, suggesting that common neurocircuits co-regulate stress and feeding. Our tracing studies indicate that non-AgRP GABAergic neurons in the arcuate

nucleus (Arc, denoted as Arc^{GABA} neurons) send direct inputs to corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVH, denoted as PVH^{CRH} neurons). While PVH^{CRH} neurons exhibit time-locked activation in response to various environmental stressors, we found that both GABA release onto PVH^{CRH} neurons and Arc^{GABA} neuron activity decrease under chronic stress conditions (e.g., tail suspension and restraint), but not under acute stressors (e.g., water spray and air puff). Specifically, Arc^{GABA} neuronal activity correlates with GABA release onto PVH^{CRH} neurons, but it is anticorrelated with PVH^{CRH} neuronal activity in response to the same stressor. Furthermore, chemogenetic inhibition of PVH^{CRH}-projecting Arc^{GABA} neurons enhances PVH^{CRH} neuronal activity and elevates stress levels, phenocopying the direct activation of PVH^{CRH} neurons. Conversely, chemogenetic activation of PVH^{CRH}-projecting Arc^{GABA} neurons reduces both PVH^{CRH} neuronal activity and stress levels, and this phenotype can be completely abolished by concurrent excitation of PVH^{CRH} neurons. Altogether, our findings indicate that non-AgRP Arc^{GABA} neurons play a critical role in encoding the scalability of stress responses to environmental stressors by modulating inhibitory GABA release onto PVH^{CRH} neurons in a stimulus strength-dependent manner.

Disclosures: Y. Cao: None. **C. Su:** None. **F.H. Do Monte:** None. **Q. Tong:** None. **Y. Xu:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.08/R1

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIEHS R00ES033278 to CJS

Title: Effects of prenatal exposure to air pollution and maternal stress on social and neuroimmune gene expression in the gut-brain axis

Authors: *E. M. MARTIN[1,2], N. Y. LI[3,2], M. J. KERN[2,4], M. F. WINTERS[2], M. C. STOEHR[2], C. J. SMITH[2];
[1]Vanderbilt Univ., Nashville, TN; [2]Psychology and Neurosci., Boston Col., Chestnut Hill, MA; [3]Univ. of Pittsburgh, Pittsburgh, PA; [4]Univ. of Michigan, Ann Arbor, MI

Abstract: Air pollution and maternal stress exposure during pregnancy are known prenatal risk factors for the development of disorders characterized by alterations in social behavior, such as autism spectrum disorder (ASD). Because high levels of air pollution and maternal stress converge on the same communities, they may synergize to increase the risk of developing ASD. We model the cumulative effects of these exposures by exposing pregnant mouse dams to diesel particles (DEP) and maternal stress (MS). Previous work in this model has revealed male-specific changes in social behavior, microglial function, and gut microbiome composition. Yet, it

remains unclear a) what social neuropeptide systems are disrupted in the brain to mediate these effects and b) what shifts in the intestinal epithelium may accompany alterations in the gut microbiome. To address these questions, we collected tissue punches from 6 brain regions in the social behavior neural network and from both the small (jejunum and ileum) and large (colon) intestine. These brain regions included the nucleus accumbens (NAc), bed nucleus of the stria terminalis (BNST), lateral septum (LS), amygdala (AMY), dorsal hippocampus (dHIP) and ventral hippocampus (vHIP). Using qPCR, we assessed expression of neuropeptide receptor genes, genes related to neuroimmune function, genes related to dopaminergic transmission, and genes related to peripheral immune function. Overall, of the brain regions evaluated, we found that DEP/MS most consistently affected gene expression in the NAc and dHIP. DEP/MS increased vasopressin receptor 1A (V1aR) expression in the NAc in both sexes but did not affect oxytocin receptor (OTR) expression in the brain. It also increased expression of neuroimmune-related genes in the NAc and dHIP in both sexes. In the NAc, but not the dHIP, DEP/MS had sex-specific effects on dopamine receptor 2 expression and upregulated expression of genes related to dopamine metabolism and synaptic reuptake. In the intestines, DEP/MS decreased OTR and V1aR expression in males and had region-specific effects on these genes in females. It also tended to increase expression of genes related to peripheral immune function in the intestines in females and decrease expression of these genes in males. These data demonstrate that, despite previous work suggesting that males are more vulnerable to the effects of DEP/MS than females, DEP/MS affects expression of genes related to social behavior and neuroimmune function in both sexes. Additionally, DEP/MS likely alters dopaminergic signaling in the NAc. Finally, these data suggest a novel role for intestinal OTR and V1aR signaling in the etiology of ASD.

Disclosures: E.M. Martin: None. **N.Y. Li:** None. **M.J. Kern:** None. **M.F. Winters:** None. **M.C. Stoehr:** None. **C.J. Smith:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.09/R2

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIEHS R00ES033278

Title: Microglial interactions with synapses on oxytocin receptor-expressing neurons in the anterior cingulate cortex following combined prenatal exposure to air pollution and maternal stress

Authors: *M. C. STOEHR, J. T. BABALOLA, J. XUE, C. J. SMITH;
Boston Col., Chestnut Hill, MA

Abstract: The ability to navigate social relationships and hierarchies is critical to both survival and well-being, across species. The neuropeptides oxytocin (OXT) and vasopressin (AVP) are critical modulators of social behavior and their receptors, oxytocin receptor (Oxtr) and vasopressin 1a receptor (V1aR), respectively, are highly expressed throughout the social brain. Many neurodevelopmental and neuropsychiatric disorders are characterized by deficits in social behavior. Furthermore, many environmental contributors to these disorders - such as air pollution and maternal stress - disrupts social behavior. Our lab utilizes a mouse model of prenatal exposure to diesel exhaust particles (DEP) and maternal stress (MS) and we have found that DEP/MS induces social behavior deficits in male but not female offspring. However, whether DEP/MS exposure alters OXT and/or AVP systems is unknown. Here, we first assessed the expression of OXT and AVP cell bodies and fibers within the hypothalamus in late adolescence. Interestingly, we found no effects of DEP/MS on the density of OXT or AVP cell bodies or fiber projections in the hypothalamus. However, we observed a trend towards a decrease in Oxtr expression in the anterior cingulate cortex (ACC) of male offspring following DEP/MS as compared to control ($p=0.05$). Microglia, the resident immune cells of the brain, are known to be crucial for synaptic elimination and refinement in the developing brain, particularly during adolescence. Additional work in the DEP/MS model has demonstrated disrupted microglial gene expression, phagocytic function, and synaptic development in the ACC of male offspring during early postnatal development. We hypothesize that prenatal exposure to DEP/MS increases microglial elimination of synapses on Oxtr+ neurons in the ACC in male but not female offspring. To test this hypothesis, we are quantifying Oxtr+ cell number in the ACC across cortical layers using a tdTom-Oxtr fluorescent reporter, as well as microglial interactions with synapses on Oxtr+ neurons in the ACC during adolescence using Imaris 3D reconstruction software. Together, this work suggests that DEP/MS disrupts Oxtr expression, but not OXT or AVP cell/fiber density and highlights a potential role for microglia in the mediating these effects.

Disclosures: M.C. Stoehr: None. **J.T. Babalola:** None. **J. Xue:** None. **C.J. Smith:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

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Program #/Poster #: PSTR079.10/R3

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NIEHS R00ES033278

Title: Alterations in oxytocin and vasopressin systems in the developing gut-brain axis following prenatal exposure to air pollution and maternal stress: a role for milk

Authors: *A. P. LALLY[1], A. DJERDJAJ[1], E. M. MARTIN[2,1], C. J. SMITH[1];
[1]Boston Col., Chestnut Hill, MA; [2]Vanderbilt Univ., Nashville, TN

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by social deficits and repetitive behavior with higher diagnosis rates in males. Air pollution and maternal stress are two prenatal risk factors for ASD that often converge on the same populations. We model the potential cumulative effects of these risk factors by exposing pregnant dams to diesel exhaust particles (DEP) and resource deprivation-based maternal stress (MS) during gestation; previous work in this model finds social deficits, changes to microglia morphology and gene expression, and alterations in the gut microbiome of male but not female offspring. Oxytocin (OXT) and vasopressin (AVP) are neuropeptides implicated in social behavior and OXT specifically is widely understood to have a vital role in maternal care/lactation and social development. Previous work in the DEP/MS model found that behavioral, microglial, and microbial changes in males were rescued by cross-fostering DEP/MS offspring to a vehicle/control (VEH/CON) dam at birth, pointing to maternal milk as a protective factor. Here, we investigated maternal milk as a crucial contributor to infant gut and brain development and alterations in the OXT/AVP systems in brain and intestinal tissue of offspring. Milk was collected from DEP/MS and VEH/CON dams on three days between P10-14, corresponding with the peak lactation period. A cohort of offspring (avg 2M/2F per litter) were collected the day following final milk collection from a given dam (P13-15); tissue from all gut regions, cecum contents, and fetal brains were flash frozen. We found a significant difference in volume of milk collected between groups ($p=0.0382$) with DEP/MS dams demonstrating lower milk letdown, and a trend towards lower OXT concentration in milk of DEP/MS dams ($p=0.0712$). qPCR in offspring intestinal samples shows a significant decrease in V1AR expression in ileum DEP/MS samples ($p=0.0441$) and a trend towards a treatment effect in OTR expression in ileum ($p=0.0506$), with OTR expression significantly lowered in DEP/MS males specifically ($p=0.0163$). Initial results related to immune genes shows a significant decrease in TLR4 expression in DEP/MS offspring in the ileum ($p=0.0018$) and a trend towards an interaction between sex and treatment in the colon ($p=0.0670$), with DEP males demonstrating lower expression, and DEP females, higher expression, than their VEH/CON counterparts. Currently, we are investigating expression of OXT and its receptor at the mRNA and protein levels in brains of the same cohort of offspring using a combination of RNAscope and IHC to further characterize the gut-brain axis at this early developmental timepoint.

Disclosures: A.P. Lally: None. **A. Djerdjaj:** None. **E.M. Martin:** None. **C.J. Smith:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.11/R4

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: Psychopharmacology Lab Research Fund

Title: Analysis of an animal model of gender affirming testosterone therapy in adolescent and adult female rats

Authors: A. R. CALVERT[1], F. SPENCER[1], R. M. CRAFT[2], ***M. M. MORGAN**[1];
[1]Washington State Univ. - Vancouver, Vancouver, WA; [2]Psychology, Washington State Univ., Pullman, WA

Abstract: The gonadal steroid hormone testosterone has major effects on biology and behavior. These effects raise questions about the potential health benefits and costs of gender affirming testosterone (T) therapy. Surprisingly little basic research has been done examining how the effects of T might vary depending on the age when such therapy begins. The present study was conducted to expand our previous research validating an animal model of gender affirming hormone therapy by assessing the effect of continuous T administration on adolescent and adult female rats. Blank or T-filled capsules were implanted subcutaneously into gonadally intact adolescent (post-natal day 43) and adult (post-natal day 92) female rats. T treatment at male-typical levels suppressed estrous cycling and uterine weight, increased body weight and lean mass, and increased low density lipoprotein levels in both adolescent and adult rats - effects consistent with defeminization and masculinization of the rats. T also increased clitoral gland size in adolescent, but not adult rats. Given that female rats tend to be more sensitive than males to delta-9-tetrahydrocannabinol (THC), the primary psychoactive compound in cannabis, the impact of acute administration of THC (0.32, 1.0, or 3.2 mg/kg, s.c.) was assessed. THC dose dependently decreased voluntary home cage wheel running in adolescent and adult female rats whether they were receiving T or not. Administration of 3.2 mg/kg of THC produced a near complete inhibition of wheel running for over 12 hrs. These results suggest that T exposure in gonadally intact adolescent or adult female rats is a valid model of gender affirming T therapy in terms of the impact of T on a variety of physiological measures. In contrast, T did not reduce wheel running activity in female rats to a level previously observed in males. These effects were consistent whether administration of T was initiated during adolescence or adulthood.

Disclosures: A.R. Calvert: None. **F. Spencer:** None. **R.M. Craft:** None. **M.M. Morgan:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR079.12/R5

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: NSERC Discovery Grant RGPIN 2016 06257

Title: Insulin modulates glutamate transmission in the rat dorsomedial hypothalamus

Authors: *K. M. CROSBY, C. LAUREIJS;
Biol., Mount Allison Univ., Sackville, NB, Canada

Abstract: Insulin, an important regulator of peripheral blood glucose levels, can also act in the brain to influence energy metabolism. The dorsomedial hypothalamus (DMH) contains appetite-regulating neurons that express insulin receptors, but nothing is known about the effects of

insulin on synaptic function in this region. To determine the effect of insulin on glutamate transmission in the DMH, we used whole-cell patch clamp electrophysiology to examine glutamatergic currents and action potentials in DMH neurons of young male and female Sprague-Dawley rats. Insulin decreased neuronal excitability in both sexes by reducing action potential frequency and amplitude. Insulin also decreased evoked glutamate current amplitude, with variance analysis suggesting that insulin postsynaptically affected quantal release. Both insulin receptors and insulin-like growth factor-1 receptors were required for this effect, suggesting that these receptors may act in a synergistic manner. Interestingly, only insulin receptors mediated the tonic effects of insulin on glutamate transmission; evoked current amplitude and spontaneous current frequency were increased in the presence of an insulin receptor antagonist. We also found evidence of a sexually dimorphic downstream pathway from both receptors. Finally, the effects of insulin on glutamate currents appear to be blocked in animals that have been fasted for 24 hours, suggesting that insulin may act in the DMH to suppress appetite. Overall, our data suggest that insulin modulates glutamate transmission in the DMH to potentially influence appetite. This research contributes to our understanding of how insulin acts in the hypothalamus to alter synaptic function, with potential implications for diabetes and obesity.

Disclosures: K.M. Crosby: None. **C. Laureijs:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.13/R6

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Development of prairie vole (Microtus ochrogaster) hypothalamic atlas using light-sheet microscopy (3D), NeuN immunofluorescence.

Authors: A. COLE[1], A. PEREZ[2], C. Y. PUERTA[1], *B. S. CUSHING[3];
[1]Univ. of Texas at El Paso, El Paso, TX; [2]The Univ. of Texas at El Paso, El Paso, TX; [3]The Univ. of Texas At El Paso, El Paso, TX

Abstract: Prairie voles (*Microtus ochrogaster*), which are socially monogamous, are the primary rodent model for studying the neural regulation of prosocial behavior and the expression of high levels of affiliative behavior. They are also a translational model because of the similarities in social behavior, pair bond formation, and biparental care to humans. However, despite the critical role they have play in social and behavioral neuroscience, there is currently no comprehensive 3D prairie vole brain atlas. Therefore, we are developing a detailed 3D digital atlas of the hypothalamus, an area known for regulating behavior and emotional response, to understand the structural detail of the nuclei and subregions that modulate prairie vole behaviors. We have done this using IMARIS software, a NeuN (Neuronal Nuclei) antibody-stained prairie vole brain, and previously acquired functional MRI data to overlap and parcel the hypothalamus

to create a 3D prairie vole atlas. By mapping the cytoarchitectures that underlie neurobiological mechanisms in prairie voles, we are providing a novel hypothalamic 3D model for future studies on social deficit disorders and affective disorders, through a structural tool set that will support current and future researchers using this important model system.

Disclosures: A. Cole: None. **A. Perez:** None. **C.Y. Puerta:** None. **B.S. Cushing:** None.

Poster

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.14/R7

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Intraspecific oxytocinergic neuron expression in behaviorally distinct prairie voles (Microtus ochrogaster): A 3D light-sheet imaging approach

Authors: *A. B. PEREZ[1], C. Y. PUERTA[3], A. P. COLE[3], Y. SALCIDO[1], S. BALIVADA[2], B. S. CUSHING[4];
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Abstract: Oxytocin is produced in three brain regions: the paraventricular nucleus (PVN), the supraoptic nucleus (SON), and the mammillary body (MBB). A large body of work has shown that oxytocinergic neurons in the PVN, and perhaps the MBB, play a critical role in the expression of prosocial behaviors, including the formation of partner preferences, long-term pair bonds, and parental behavior. Prairie voles (Microtus ochrogaster), a socially monogamous species, are the primary rodent model for studying the central effects of oxytocin from the PVN in regulating the expression of high levels of affiliative behavior. In addition to their behaviors being similar to humans, prairie voles provide a unique model for investigating the neurobiology of social behaviors because the degree of expression of behaviors associated with social monogamy differs geographically. Prairie voles from Illinois (IL), which have been used in the vast majority of studies, display significantly higher levels of prosocial behavior and lower levels of aggression than prairie voles from Kansas (KS). Further, these differences are enhanced in hybrids, as KI (KS mothers and IL fathers) males display the lowest levels of prosocial behavior and the highest levels of aggression. Based upon behavioral expression and known neuroanatomical 2D expression, we hypothesized that intraspecific differences are associated with differential expression of oxytocinergic neurons, especially in the PVN. Using LifeCanvas Technologies™ methodology, we cleared brains from IL, KS, KI, and IK males and females. Then, we immunolabeled oxytocin and visualized the expression of oxytocinergic neurons using 3D light-sheet microscopy in the PVN, SON, and MBB. Preliminary results supported our hypothesis, with significant differences in the expression of oxytocinergic neurons in the PVN between females and males and the MBB in only males. These findings suggest that intraspecific differences in the expression of oxytocinergic neurons in selected nuclei are associated with

differential expression of prosocial behavior in prairie voles, and that the MBB may play a greater role in males than females.

Disclosures: A.B. Perez: None. **C.Y. Puerta:** None. **A.P. Cole:** None. **Y. Salcido:** None. **S. Balivada:** None. **B.S. Cushing:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.15/R8

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: 3d light-sheet imaging: differential expression of vasopressinergic neurons between behaviorally distinct prairie vole (microtus ochrogaster) populations.

Authors: *C. Y. PUERTA[1], A. B. PEREZ[2], A. P. COLE[1], S. BALIVADA[3], B. S. CUSHING[4]; [1]Univ. of Texas at El Paso, El Paso, TX; [3]Biol. Sci., [2]The Univ. of Texas at El Paso, El Paso, TX; [4]Biol. Sci., The Univ. of Texas At El Paso, El Paso, TX

Abstract: Arginine vasopressin (AVP) is a neuropeptide critically involved in the regulation of social behavior, stress response, and water retention. In the brain, AVP is primarily produced in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. In socially monogamous species, such as the prairie vole (*Microtus ochrogaster*), AVP is associated with aggressive behaviors, mate guarding, and social bonding. To investigate neural underpinnings of behavioral variation, this study uses prairie voles from two geographically distinct populations: Illinois (IL), which exhibit high levels of prosocial behavior, and Kansas (KS), which exhibit more solitary behavior and increased aggression. Using tissue clearing techniques from LifeCanvas Technologies, followed by whole-brain 3D light-sheet fluorescence microscopy, we visualize AVP-expressing neurons across intact brains with high spatial resolution. Light-sheet microscopy preserves three-dimensional brain architecture, allowing for insight into the shape, size, and cellular density of vasopressinergic regions. Given the behavioral differences between populations, we hypothesize that KS voles will have a higher number of vasopressinergic neurons in the PVN and SON compared to IL voles. These findings will not only enhance our understanding of how AVP contributes to natural variation in sociality and aggression but also highlight the impact of modern 3D imaging techniques in advancing behavioral neuroscience.

Disclosures: C.Y. Puerta: None. **A.B. Perez:** None. **A.P. Cole:** None. **S. Balivada:** None. **B.S. Cushing:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.16/R9

Topic: G.02. Neuroendrocrine Processes and Behavior

Title: Cross-species divergence in social brain connectivity through graph theory analysis of rats and mice

Authors: I. MURILLO[1], P. P. KULKARNI[2], C. F. FERRIS[3], B. S. CUSHING[4], ***R. J. ORTIZ**[1]; [1]Psychology, Northern Illinois Univ., DeKalb, IL; [2]Psychology, [3]Dept Psychology, Northeastern Univ., Boston, MA; [4]Biol. Sci., The Univ. of Texas At El Paso, El Paso, TX

Abstract: Understanding similarities and differences in brain network connectivity across species is crucial for translating findings from animal to human models, particularly concerning complex behaviors such as sensory-social processing. We used graph theory to quantify neural connectivity from resting-state fMRI data, focusing on centrality metrics like degree and hub analysis. Previous research identified a group of neural regions associated with prosocial behavior (PS core) and olfactory integration (O core) and examined significant differences in global, inter- and intra-core connectivity between two prosocially distinct prairie vole populations. The more prosocial male prairie voles had significantly greater connectivity and the presence of neural hubs within the combined prosocial-olfactory (PS + O) core. In this study, we evaluated the generalizability of these findings by comparing connectivity patterns and hub expression in male rats and mice, species characterized by polygynous rather than socially monogamous reproductive strategies. We hypothesized that rats and mice would demonstrate low connectivity and a lack of hubs within the PS core and PS + O core. Utilizing graph theory analysis of resting-state fMRI data, we found that male rats and mice did not show significant differences in PS core or PS + O core connectivity degree centrality metrics. However, both groups displayed divergent patterns of PS + O core connectivity hubs. These results suggest that while prosocial and olfactory networks are conserved in rats and mice, their functional integration diverges, potentially reflecting species-specific social strategies.

Disclosures: I. Murillo: None. **P.P. Kulkarni:** Other; Ekam Solutions. **C.F. Ferris:** Other; Ekam Solutions. **B.S. Cushing:** None. **R.J. Ortiz:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.17/R10

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: Secihti CF-2023-G-243
PAPIIT-UNAM IT200125

Title: Parabrachial/kf-amygdala peptidergic circuit: a new role in cardiovascular regulation

Authors: *L. ZHANG[1], V. S. HERNANDEZ[2];
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Abstract: Neuroendocrine signaling within the cardiovascular-respiratory-brain axis remains poorly understood. The Kölliker-Fuse nucleus is a dorsolateral subregion of the parabrachial complex, residing at the pontine-mesencephalic junction. Traditionally viewed as part of the respiratory and autonomic network, recent evidence has drastically expanded its functional relevance. Historically, the KF nucleus is known for its pivotal role in respiratory rhythm modulation, autonomic regulation, and chemoreception. It coordinates phase transitions in the respiratory cycle and interfaces with medullary centers to shape breathing behavior. Recent findings from our group have identified a novel population of large, calyx of Held-like axo-somatic terminals in the capsular subdivision of the central amygdala (CeC). These specialized terminals originate from glutamatergic/cholinergic neurons in the Kölliker-Fuse (KF) region of the parabrachial complex and co-express PACAP, CGRP, VAChT, VGluT1, and VGluT2. They selectively target PKCδ+ neurons in the CeC that co-express GluD1, forming a highly specialized peptidergic relay. To test the physiological activation of this circuit, we administered systemic hydralazine (25 mg/kg, i.p.) to induce acute hypotension and analyzed c-Fos expression 90 minutes later. Compared to controls, treated animals showed robust Fos induction in the KF/PBN, CeC, and hypothalamic nuclei including the PVN and SON. Notably, a subset of Fos+ neurons in the KF co-expressed calretinin (CR), a marker previously associated with the PACAP/CGRP projection to the CeC. Additionally, CR+ ring-like structures were observed encircling Fos+/PKCδ+ neurons in the CeC. These results suggest that acute cardiovascular perturbation activates a KF→CeC→hypothalamus peptidergic circuit, likely involved in viscerosensory processing and neuroendocrine adaptation. Our findings expand the functional scope of the KF beyond respiratory control, implicating it in cardiovascular regulation via high-fidelity telencephalic projections. This work extends our previous description of multimodal calyceal synapses in the amygdala and provides new insights into brainstem-amygdala-hypothalamic communication in homeostatic control.

Disclosures: L. Zhang: None. **V.S. Hernandez:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.18/R11

Topic: G.02. Neuroendrocrine Processes and Behavior

Support: CREATE-NSERC Fellowship
BBDC
NSERC Discovery Grant

Title: Role of Arcuate GLP-1R Neurons in Energy and Glucose Homeostasis

Authors: *I. SINGH[1], J. FENG[2], L. WANG[3], Z. P. PANG[4], M. WHEELER[2];
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Abstract: Background: The central nervous system (CNS) plays a crucial role in regulating metabolism, integrating hormonal and nutrient signals to control feeding and glucose homeostasis. Glucagon-like peptide-1 (GLP-1), produced by neurons in the nucleus tractus solitarius (NTS), exerts central effects via its receptor (GLP-1R), which is widely expressed in the brain. The arcuate nucleus (ARC) of the hypothalamus expresses dense GLP-1R and receives projections from NTS GLP-1+ neurons, suggesting it may act as a central hub for metabolic regulation. While GLP-1 receptor agonists (GLP-1RAs) show clinical efficacy in treating type 2 diabetes and obesity, the underlying CNS mechanisms remain poorly defined.
Objectives: This study aims to dissect the functional role of ARC GLP-1R-expressing neurons in regulating energy intake, insulin secretion, hepatic glucose metabolism, and brain-pancreas connectivity.
Methods: To selectively manipulate ARC GLP-1R neurons, Cre-dependent adeno-associated viruses (AAVs) encoding excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs were injected into the ARC of *GLP-1R-ires-cre* mice. Neuronal activity was modulated via clozapine-N-oxide (CNO). Mice were phenotyped for food intake, glucose tolerance (GTT), insulin secretion, and insulin sensitivity (ITT). Pharmacological studies with Exendin-4 (GLP-1R agonist) and Exendin-9 (antagonist) validated central GLP-1R function. Hepatic tissue was analyzed for metabolic gene expression. Pseudorabies virus (PRV) tracing was used to map ARC GLP-1R neuron projections to the pancreas.
Results: Activation of ARC GLP-1R neurons suppressed food intake and enhanced insulin secretion, while inhibition increased feeding and led to glucose intolerance. These effects were recapitulated by Exendin-4 and blocked by Exendin-9, supporting a functional role of GLP-1R signaling in the ARC. Hepatic gene analysis revealed that activation decreased expression of *Pepck*, *G6pc*, and *Cpt1a*, and increased *Ppargc1a*, indicating improved hepatic insulin sensitivity. Tracing confirmed direct neuronal connectivity between ARC GLP-1R neurons and the pancreas, supporting a brain-islet regulatory axis.
Conclusions: Together, these findings identify ARC GLP-1R neurons as integrators of central and peripheral metabolic control. They regulate appetite, pancreatic function, and hepatic metabolism via coordinated neural and hormonal pathways. Future studies employing spatial transcriptomics and insulin signaling assays will define their molecular heterogeneity and systemic impact.

Disclosures: I. Singh: None. **J. Feng:** None. **L. Wang:** None. **Z.P. Pang:** None. **M. Wheeler:** None.

Poster

PSTR079: Neuroendocrine Systems: Anatomy and Physiology I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR079.19/R12

Topic: G.08. Food and Water Intake and Energy Balance

Support: Intramural research program at National Institutes of Health.

Title: Hunger-state-dependent regulation of Sytβ in insulin-producing cells alters starvation responses

Authors: *G. YANG[1], H.-L. CHEN[2], Q. YUAN[3];
[1]NIH, Natl. Inst. of Neurolog. Disorders & Stroke (NINDS), Washington, DC; [2]NINDS, NIH, Bethesda, MD; [3]NINDS, NIH/NINDS, BETHESDA, MD

Abstract: The neural underpinnings of the internal nutritional state and its influence on animal behavior remain incompletely understood. Hormones and neuropeptides, such as the mammalian Insulin and insulin-like growth factor-1 (IGF-1), or the insulin-like peptides (ILPs) in *Drosophila*, serve as key neuromodulatory signals that regulate hunger-induced feeding behaviors. To uncover the molecular machinery responsible for state-dependent regulation of feeding, we investigated the signaling pathways controlling ILP release from the *Drosophila* Insulin-Producing Cells (IPCs). Specially, we examined the role of Synaptotagmin-beta (Sytβ), a member of synaptotagmin family that is highly enriched in *Drosophila* neuroendocrine cells and functions as an inhibitory regulator of dense-core vesicle release (*in preparation*). Remarkably, we found that Sytβ levels are upregulated in IPCs following fasting. Flies lacking Sytβ ($Syt\beta^{-/-}$) exhibited increased sensitivity to starvation, succumbing more rapidly than controls, and showed reduced food intake, indicating a blunted hunger response. Furthermore, IPC-specific manipulations of Sytβ strongly influenced hunger-induced feeding, highlighting its crucial modulatory role in ILP release. Together, our findings reveal how a specific synaptotagmin isoform integrates hunger state with neuropeptide signaling to shape adaptive responses to nutrient availability. More broadly, this work highlights disinhibition as a key mechanism in state-dependent neuromodulation.

Disclosures: G. Yang: None. **H. Chen:** None. **Q. Yuan:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.01/R13

Topic: G.03. Stress and the Brain

Title: Impact of caffeine on participant memory for a stressful experience and the development of intrusive memories

Authors: *J. L. KIRKLAND[1], K. R. MCCLENEN[1], N. E. PORTER[1], S. M. WOOD[1], J. GROSS[1], M. E. PAPHANCHITH[1], K. M. SIEREVELD[1], J. I. C. IFEAKANWA[1], C. M. GURGANUS[1], K. N. SUZELIS[1], A. J. FARRELL[1], B. R. RORABAUGH[2], P. R. ZOLADZ[1]; [1]Psychology & Educ., Ohio Northern Univ., Ada, OH; [2]Pharmaceut. Sci., Marshall Univ., Huntington, WV

Abstract: Stressful experiences can narrow our attention, which enhances memory for central details of the experience. These effects result from an interaction between glucocorticoids and norepinephrine in cognitive brain regions. However, the impact of stress on memory can be influenced by multiple factors related to individuals, including pharmacological agents they ingest. Most individuals consume caffeine, the most widely used psychotropic, on a daily basis. Research has shown that caffeine stimulates the release of glucocorticoids and catecholamines and can enhance cognition. Caffeine can also amplify stress-induced increases in arousal and aggravate PTSD-like symptoms in animal models. Thus, we examined the impact of caffeine on memory for a stressful experience and the development of intrusive memories related to that experience. Undergraduate students ingested 200 mg caffeine or a placebo. Thirty minutes later, they were exposed to a modified version of the Trier Social Stress Test (TSST) or the friendly-TSST (f-TSST), both of which were designed to enable participant memory for the experiences to be quantified. The TSST required participants to deliver a ten-minute speech in front of two lab panel members as part of a mock job interview; the f-TSST required participants to casually converse with panel members about their interests and hobbies. In both conditions, the panel members interacted with (central) or did not interact with (peripheral) several objects sitting on a desk in front of them. Subjective measures of alertness and anxiety were collected, and saliva samples were collected to assay for cortisol and alpha-amylase. The next day, participants' memory for the objects that were present on Day 1 was assessed. We also quantified participants' intrusive memories on Days 2, 4, 6, and 8. Consistent with previous work, the TSST group reported greater subjective anxiety than the f-TSST group, and both the TSST and f-TSST manipulations resulted in increased salivary alpha-amylase. The TSST group exhibited greater recall of central objects and fewer falsely recalled objects than the f-TSST group. Interestingly, caffeine led to reduced recall of central objects, relative to placebo, an effect that was observed only in f-TSST participants. TSST-exposed participants reported intrusive memories that were more intense, bothersome, and upsetting than those reported by f-TSST participants. Caffeine did not impact any measure of intrusive memories. Overall, our findings suggest that caffeine, alone, may reduce memory for central details of an experience, but has minimal impact on what is remembered about a stressful event.

Disclosures: J.L. Kirkland: None. **K.R. McClenen:** None. **N.E. Porter:** None. **S.M. Wood:** None. **J. Gross:** None. **M.E. Paphanchith:** None. **K.M. Siereveld:** None. **J.I.C. Ifeakanwa:** None. **C.M. Gurganus:** None. **K.N. Suzelis:** None. **A.J. Farrell:** None. **B.R. Rorabaugh:** None. **P.R. Zoladz:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.02/R14

Topic: G.03. Stress and the Brain

Support: Korea Research Foundation Grant/(RS-2024-00440371

Title: Ketamine treatment rescues memory impairments induced by uncontrollable stress

Authors: *S. PARK[1], J.-S. HAN[2];
[2]Biol. Sci., [1]Konkuk Univ., Seoul, Korea, Republic of

Abstract: Major depressive disorder (MDD) is a prevalent and debilitating psychiatric condition characterized by persistent sadness, hopelessness, and loss of interest. While conventional antidepressants such as selective serotonin and norepinephrine reuptake inhibitors (SSRIs/SNRIs) are widely prescribed, they typically require prolonged administration (weeks to months) to achieve therapeutic effects, and a significant proportion of patients fail to respond adequately. Recently, ketamine, an N-methyl-D-aspartate receptor (NMDAR) antagonist, has gained considerable attention for its rapid and sustained antidepressant effects, even after a single dose. Given that stress is a major precipitating factor in depression and is commonly used to induce depressive-like behaviors in animal models, understanding the interaction between ketamine and stress is critical. Research into the mechanisms underlying ketamine's antidepressant effects is extensive. Yet, the association between ketamine and stress remains poorly understood. In this study, we investigated the effects of ketamine on stress-induced cognitive impairment in rats. We employed a 60-minute restraint and 60 intermittent tail shocks ("60-min stress") to induce cognitive impairment based on our previous findings. Cognitive status was assessed using the novel object recognition (NOR) task. Control rats administered saline (10 mg/kg, i.v.) exhibited significant memory impairment following the 60-min stress exposure. In contrast, ketamine-treated rats did not show memory impairments, suggesting a protective effect against stress-induced cognitive impairment. These results indicate that intravenous ketamine administration effectively reverses stress-induced cognitive impairment and may offer therapeutic potential for treating cognitive deficits associated with depression. Supported by the Korea Research Foundation Grant funded by the Korean Government (RS-2024-00440371 to Jung-Soo Han).

Disclosures: S. Park: None. **J. Han:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.03/R15

Topic: G.03. Stress and the Brain

Title: Sex differences in memory and serum biomarkers following folic acid-induced acute kidney injury in rats

Authors: *D. FLORES GUZMAN, J. M. IBARRA HERNÁNDEZ, M. PERALES-QUINTANA;
Fisiologia. Facultad de Medicina. UANL, Monterrey, Mexico

Abstract: Acute kidney injury (AKI) is a serious condition associated with high mortality, commonly occurring in hospitalized patients—particularly after surgeries or diagnostic procedures—and more frequently in older adults with comorbidities. One of the consequences of AKI is the development of uremic syndrome, whose systemic effects include neurological alterations such as cognitive impairment. However, sex-related differences in the cognitive impact of this syndrome remain poorly characterized. This study aimed to evaluate whether folic acid-induced AKI affects short- and long-term spatial memory in male and female rats. Male and female Wistar rats were assigned to three groups: (1) control, (2) AKI before training, and (3) AKI after training. AKI was induced by a single intraperitoneal dose of folic acid (250 mg/kg). Spatial memory was assessed using the Barnes maze after four days of training, followed by a short-term memory test on day 5 and a long-term memory test on day 12. Plasma levels of ALT, BUN, creatinine, alkaline phosphatase, glucose, amylase, phosphate, and potassium were measured at baseline, and on days 7 and 12 post-injury, along with 24-hour urinary volume. In male rats, plasma levels of BUN (10.25 ± 1.43 mM), alkaline phosphatase (536 ± 200 U/L), ALT (73.25 ± 15.5 U/L), amylase (335 ± 56.3 U/L), and potassium (6.55 ± 0.66 mM) remained elevated at days 7 and 12 compared to baseline. In contrast, females exhibited a significant increase in urinary volume on day 12 (30 ± 8 mL/24 h) compared to their baseline values and to males (10 ± 3 mL/24 h). In both sexes, animals with renal injury prior to training showed a significant reduction ($P < 0.05$) in escape latency during the short-term memory test, coinciding with elevated levels of BUN, alkaline phosphatase, ALT, amylase, and potassium. The reduced latency suggests that AKI-induced metabolic changes may modulate neurotrophic factors that enhance memory. Furthermore, the observed sex differences may reflect greater damage persistence in males, whereas increased diuresis in females may represent a protective adaptive mechanism.

Disclosures: D. Flores Guzman: None. **J.M. Ibarra Hernández:** None. **M. Perales-quintana:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.04/R16

Topic: G.03. Stress and the Brain

Support: R01 MH136708

Title: Sex Differences in the Effects of Chronic Stress, Chronic Corticosterone and Prefrontal Cortical REDD1 Overexpression on Pavlovian Autoshaping Behavior in Rats

Authors: *B. KURTOGLU[1], D. R. OLIVEIRA[1], M. K. ESTES[1], E. THOMPSON[1], D. S. WHEELER[2], M. C. HEARING[2], R. A. WHEELER[2], J. R. MANTSCH[1];
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Abstract: Approach motivation is stemming from the expectation of reward and is classified in the Research Domain Criteria (RDoC) matrix under the positive valence domain. Deficits in approach motivation have been linked to neuropsychiatric disease states such as depression and addiction and result in reduced resilience to negative life events like stress. The prefrontal cortex (PFC) is important for regulating approach motivation behavior and chronic stress is known to produce structural and functional changes in PFC that might also contribute to disease states. To study the changes in approach motivation, we used an operant-based Pavlovian autoshaping (PAS) task where a cue, the presentation of a lever is associated with the subsequent delivery of a sucrose pellet reward. Rats express the learned association between the lever and food delivery by approaching the lever. We find that chronic unpredictable stress (CUS) produces deficits in PAS task, by attenuating CS+ approach in male ($p=0.002$) and female ($p=0.031$) rats. However, what neural adaptations drive these deficits remain unclear. To gain more insight into this, our studies focus on the protein REDD1 (regulated in development and DNA damage responses-1) as it is increased in post-mortem PFC tissue from individuals diagnosed with depression. In line with these findings, we observed that there is an increase in REDD1 expression ($p=0.0001$) and a decrease in Raptor phosphorylation ($p=0.002$), one of the key elements of the mTORC1 complex, in the rat PFC after CUS, suggesting disrupted mTORC1 function. To determine if REDD1 overexpression is sufficient to produce deficits in PAS, we used a viral vector to overexpress REDD1 in the PFC of male rats before training. Similar to CUS, REDD1 overexpressing rats exhibited reduced anticipatory approach to the cue ($p<0.0001$). Because corticosterone (CORT) plays an important role in many of the adaptive responses to stress observed in laboratory experiments, we examined whether a chronic CORT elevation is sufficient to elevate REDD1 expression or reduce conditioned approach. Similar to CUS, we observed an increase in REDD1 expression and a decrease in Raptor phosphorylation after oral chronic CORT elevation. Furthermore, CORT treated male rats showed deficits in PAS, evidenced by reduced CS+ approach ($p=0.005$). However, this deficit was not observed in female rats. The observation that CUS, REDD1 overexpression and chronic CORT elevation produce deficits in approach motivation likely has relevance for understanding a number of stress related disorders. Future research will study effects of REDD1 overexpression in females and necessity of disrupted mTORC1 for stress effects.

Disclosures: B. Kurtoglu: None. **D.R. Oliveira:** None. **M.K. Estes:** None. **E. Thompson:** None. **D.S. Wheeler:** None. **M.C. Hearing:** None. **R.A. Wheeler:** None. **J.R. Mantsch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Promentis Pharmaceuticals.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.05/S1

Topic: G.03. Stress and the Brain

Title: The effects of propranolol on stress-induced spatial learning and memory performance

Authors: *G. H. KORN, J. A. SCHROEDER;
Neurosci. Program, Connecticut Col., New London, CT

Abstract: Day-to-day memory impairments of emotionally neutral material are a common complaint of individuals with post-traumatic stress disorder (PTSD). These impairments are especially pertinent, as reduced hippocampal volume and activity are frequently observed in individuals with the diagnosis. Much research has been devoted to pharmacological treatments of PTSD; however, there is much less research devoted to finding potential pharmacological treatments to prevent PTSD development following a traumatic event. Despite the limited research, studies have found that when administered following a traumatic event, the beta blocker propranolol led to lower scores on the Clinician-Administered PTSD Scale (Pitman et al., 2002). Furthermore, propranolol has been found to reduce physiological responses to subsequent mental imagery when administered post-reactivation of the traumatic event (Brunet et al., 2008). Further research into the drug has revealed that propranolol disrupts the memory consolidation process in rodents (Cohen et al., 2011). While promising, previous research leaves a need for the examination of propranolol's impact on biological factors that develop with PTSD. With this study, we aimed to investigate the effects of propranolol, when administered following psychological stress, on deficits in short and long-term memory performance. Based on previous research, we predicted that predator-odor stress-exposed Sprague-Dawley rats treated with propranolol following the exposure would have fewer short and long-term memory deficits than the control animals assessed using the Barnes maze. Comparisons of stress-exposed versus controls suggest that treatment with propranolol may affect short and long-term memory performance in specific ways. These differences reveal the importance of memory consolidation as a potentially important target for further PTSD prevention research to explore.

Disclosures: G.H. Korn: None. **J.A. Schroeder:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.06/S2

Topic: G.03. Stress and the Brain

Support: The Pritzker Consortium

Title: Genomic Effects of Stress Hormone on L-type Calcium Channels

Authors: *J. ZHANG[1], K. STANGIS[2], Y. DAS[3], G. G. MURPHY[4];
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Abstract: The World Health Organization estimates that up to 1 in every 8 individuals are impacted by psychiatric disorders with long-lasting effects on health. A major challenge in understanding these disorders and establishing robust treatments is the complicated pathophysiology. The diathesis-stress model suggests that genetic vulnerability interacts with environmental stress to promote disease development. Genome wide association studies (GWAS) have implicated *CACNA1C* and *CACNA1D*, which encode the L-type voltage gated calcium channels (LVGCCs) $Ca_V1.2$ and $Ca_V1.3$ respectively in a wide range of psychiatric disease states. To investigate the putative impact of stress on LVGCC expression, we have begun to examine LVGCC expression in hippocampal slices exposed to corticosterone (CORT). For these in vitro experiments, slices were prepared from mice in which $Ca_V1.2$ and $Ca_V1.3$ were deleted or overexpressed. Briefly, conditional $Ca_V1.2$ knockout mice were generated by crossing mice with a floxed *Cana1c* exon 2 with Syn1-cre mice for pan-neuronal deletion. Global $Ca_V1.3$ knockout mice were used in which a PGK-neo cassette was inserted into *Cana1d* exon 2. For $Ca_V1.2$ overexpression, a full-length rat Cav1.2 transgene with a hemagglutinin (HA) epitope was inserted downstream of a CAG promoter and a Lox-stop-Lox cassette. Pan neuronal expression was achieved by crossing these mice with Syn 1-cre mice. Finally, overexpression of $Ca_V1.3$ was accomplished by generating mice in which a full-length rat HA-tagged $Ca_V1.3$ transgene was cloned in downstream of the CaMKII promoter. Because of reported difficulties with probing $Ca_V1.2$ protein, a mouse line in which the endogenous $Ca_V1.2$ tagged with HA was generated via CRISPR-Cas9 editing. Hippocampal slices were incubated in increasing concentrations of CORT (100nM, 200nM, 1μM) and allowed to rest in artificial cerebral spinal fluid (aCSF) for 2 hours for genomic changes to occur before processing. Our preliminary data suggests CORT increases endogenous levels of membrane associated $Ca_V1.2$. Interestingly, male mice exhibited greater elevations in $Ca_V1.2$ protein with higher CORT concentrations (up to a 150% increase from baseline with 1μM CORT) while female mice had a less direct relationship. Rather, $Ca_V1.2$ membrane protein peaked at 50% increase from baseline with 100nM and higher CORT concentrations attenuated this response. Despite the underutilization of female mice in stress studies, there is evidence that estrogen is neuroprotective. Our initial findings further support sex differences in genomic changes in response to stress hormone. Similar experiments are ongoing using the knockout and overexpresser mice.

Disclosures: J. Zhang: None. **K. Stangis:** None. **Y. Das:** None. **G.G. Murphy:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.07/S3

Topic: G.03. Stress and the Brain

Support: CIHR - PJT-185979

Title: Uncovering chronic stress-driven changes in GABAA receptors linked to anxiety and cognitive function

Authors: *C. MEZO GONZALEZ[1], C. MARCEAU-LINHARES[1], J. CHIELLA[1], M. MARCOTTE[1], T. TOMODA[1], E. SIBILLE[2], T. D. PREVOT[3];
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Abstract: Background: Chronic stress is a major risk factor for psychiatric and brain disorders, as it disrupts the Hypothalamic-Pituitary-Adrenal (HPA) axis, leading to elevated cortisol (CORT) levels, anxiety and cognitive deficit. While the mechanisms are unclear, $GABA_A$ receptors ($GABA_AR$) play a central role in HPA axis regulation, therefore, they are a promising target for therapeutic interventions in stress-related disorders. Benzodiazepines (BZ) reduce CORT and anxiety by acting on $GABA_AR$, but their non-selective targeting of receptor subtypes (α1, α2, α3, α5) limits long-term efficacy and causes side effects such as sedation, tolerance and dependence. These limitations may stem from a poor understanding of how chronic stress affects $GABA_AR$ expression in brain regions that regulate the HPA axis. Addressing this gap could enable the development of next-generation BZ-derivatives with improved selectivity and sustained benefits.
Here, we **aim** to investigate how chronic stress alters $GABA_AR$ surface presentation and subtype distribution in the hypothalamus (HPT), prefrontal cortex (PFC), and hippocampus (HPC)—key brain regions in HPA axis control.
Methods: C57BL/6 mice were exposed to six weeks of Unpredictable Chronic Mild Stress (UCMS) to induce high CORT levels (n=16/group/sex). Weight gain and coat condition were monitored weekly. Anxiety-like behavior (Phenotyper), working memory (Y-maze), and plasma CORT (ELISA) were assessed. $GABA_AR$ surface presentation and subtype distribution in the HPT, PFC, and HPC were examined using cross-linking assays and RNAscope.
Results: UCMS led to reduced weight gain, deteriorated coat quality, increased anxiety-like behavior, impaired working memory, and elevated CORT levels ($p<0.05$) in both sexes, validating the model. Cross-linking assays showed reduced surface expression of α5- $GABA_AR$ in the HPC, a receptor critical for synaptic plasticity and memory. RNAscope revealed reduced distribution of α2- and α5- $GABA_AR$ in corticotropin-releasing hormone (CRH)-producing neurons in the HPT, where α2- $GABA_AR$ is key in anxiety regulation.
Conclusion: UCMS induces anxiety-like phenotype and increases HPA activity as expected. It also reduces α2- and α5- $GABA_AR$ expression in brain regions involved in HPA axis control, suggesting that such dynamic changes contribute to reduce BZ-efficacy. Future work will assess α1- and α3- $GABA_AR$ to further clarify subtype-specific changes, and also investigate other cell types. These findings will support the development of subtype-selective BZ-derivatives for improved treatment of stress-related psychiatric disorders.

Disclosures: C. Mezo Gonzalez: None. **C. Marceau-Linhares:** None. **J. Chiella:** None. **M. Marcotte:** None. **T. Tomoda:** None. **E. Sibille:** None. **T.D. Prevot:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.08/S4

Topic: G.03. Stress and the Brain

Support: NSERC CRD Project CRDPJ 514052-17

Title: From Struggle to Strategy: EEG Insights into Cognitive Adaptation Under Stress

Authors: *C. SU, M. ZHAO, Y. ZENG;
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Abstract: During flight simulation training, individuals adapt differently to unpredictable task demands—some stabilize their strategies over time, while others face ongoing cognitive load and fatigue. Understanding these patterns is key to improving training and safety. While stress-related brain activity has been studied, the neural basis of strategy formation over time remains unclear. This study combines subjective workload reports and EEG data to examine neurophysiological differences in adaptation. We recruited 24 healthy adults (average age 28.88, 13 female), each completing 22 pseudorandomized trials with varying difficulty. After each trial, NASA-TLX scores and 64-channel EEG were recorded. Participants were clustered into high- (n=8) and low-adaptation (n=16) groups using z-normalized NASA-TLX total scores via k-means (k=2). While the slope of workload change did not differ significantly ($p=0.65$), the low-adaptation group showed significantly greater workload volatility ($p<0.001$), suggesting reduced regulatory stability. No sex differences were found. We extracted several trial-wise EEG features, Mann-Whitney U tests were used to assess group differences across three task phases (Early: 1-7; Middle: 8-15; Late: 16-22). High-adaptation participants exhibited consistently higher approximate, sample, and permutation entropy across all phases ($p<0.001$), and higher beta/alpha ratios in Middle and Late phases ($p<0.01$). Alpha power was significantly lower in these two phases ($p<0.05$ and $p<0.001$), while theta, beta, and gamma power were lower in the Late phase ($p<0.05$). Hjorth activity was also reduced in the Middle and Late phases ($p<0.05$ and $p<0.01$). These findings reveal stable EEG-based differences in cognitive adaptation under stress. Higher complexity and reduced low-frequency power characterize more efficient neural dynamics in high-adaptation individuals. Our results suggest EEG features as potential markers for identifying adaptive strategy patterns, informing personalized training, and real-time monitoring systems.

Disclosures: C. Su: None. **M. Zhao:** None. **Y. Zeng:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.09/S5

Topic: G.03. Stress and the Brain

Title: Music, EEG and stress: connections between musicologic and EEG patterns and the mediation of physiologic stress.

Authors: *A. WARREN[1], J. A. SCHROEDER[2];
[1]Neurosci. Program, [2]Dept Psychol, Connecticut Col., New London, CT

Abstract: The interrelation between music and the brain is an emerging interdisciplinary interest within neuroscience research. Relying on both emotional connection as well as scientific properties like entrainment, this area of research explores the potential for holistic non pharmacological therapeutic interventions. Previous research has utilized music to mediate pain processing and to facilitate management, as well as musical entrainment which is the basis for this study. The purpose of this study is to examine connections between music and stress through assessment of EEG, resting heart rate (RHR), and cortisol levels. Research was conducted on the Connecticut College campus, with undergraduate student participants over three two-hour experimental periods. While employing the cold pressor test (CPT), participants were exposed to either stressful music, relaxing music, or silence with EEG and RHR data collected continuously and cortisol levels collected intermittently. EEG data from participants was then sonified and analyzed from a musical perspective and a scientific perspective. We predicted that relaxing music that was effective in reducing physiologic stress would be reflected in the recorded and sonified EEG patterns. The stressful music condition should induce higher levels of stress biomarkers for participants while undergoing CPT when compared to the no music condition. Connections and dualities between musical frequencies, specifically rhythm, and neurological frequencies are presented.

Disclosures: A. Warren: None. **J.A. Schroeder:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.10/S6

Topic: I.04. Executive Functions

Support: NIH Grant T32EB029365-04

Title: Disrupted cognitive-flexibility networks due to sickle cell disease captured by simultaneous task-based fMRI-EEG

Authors: *N. MOSSAZGHI[1], J. DISU[2], S. ROY[2], S. WOOD[2];
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Abstract: Sickle cell disease (SCD) is an inherited blood disorder caused by a single β-globin mutation. It limits oxygen delivery and disturbs neurovascular function, leading to severe complications like strokes, silent infarcts, and decline in cognitive functions. To date, no empirical evidence has examined how SCD alters the neural circuitry that supports cognitive flexibility under cognitive load. We hypothesized that SCD participants, compared with controls, would engage a wider frontoparietal control network and show weaker, delayed EEG synchronization on high-load trials—hallmarks of reduced cognitive efficiency. To investigate this, we acquired simultaneous EEG-fMRI during a digit-symbol substitution task. Ten adults [5 SCD, 34 ± 7 y; 5 controls, 25.8 ± 2.7 y] performed a digit-symbol substitution task: each trial displayed a digit-symbol pair that participants classified as congruent or incongruent with a reference table via left/right keypresses. Imaging acquisition comprised a high-resolution run (TR = 1.03 s, TE = 30 ms, 2 mm³ voxels) on a Siemens 3T Prisma. MR volumes were motion-corrected, normalized to MNI, and smoothed using fMRIPrep. The 32-channel EEG (BrainAmp, 5 kHz) was down-sampled to 250 Hz, band-passed 1-50 Hz, cleared of bad channels, and de-noised for gradient and cardio ballistic artifacts via ICA in EEGLAB. After preprocessing, voxel-wise GLMs modeled BOLD responses to congruent and incongruent trials, and mixed-effects tests contrasted these conditions between SCD and control groups. EEG was epoched (-200-1,000 ms), band-passed 1-50 Hz, Hilbert-transformed, and baseline-normalized to quantify event-related synchronization and desynchronization (ERS/ERD) in δ, θ, α, β, and γ bands followed by congruent-incongruent topographical mapping for comparison with the fMRI activations. Preliminary results show incongruent trials had longer response times in patients with SCD than controls. Furthermore, fMRI revealed these high-load trials elicited more extensive activation of frontoparietal networks in SCD than in controls, indicating greater neural effort. EEG echoed this inefficiency: patients showed delayed event-related synchronization during the P300 and N400 windows, especially over centro-parietal electrodes. Together slow response time, broader BOLD recruitment, and dampened ERS suggest individuals with SCD must engage additional cognitive-control circuitry to accomplish the same task, consistent with disrupted task-evoked. To our knowledge, this is the first simultaneous EEG-fMRI study to document alterations in cognitive flexibility and neural efficiency under cognitive load due to SCD.

Disclosures: N. Mossazghi: None. **J. Disu:** None. **S. Roy:** None. **S. Wood:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.11/S7

Topic: I.10. Human Learning and Cognition

Support: CIBERNED
MICINN
Gobierno Vasco
EITB Maratoia

Title: Reversible transient mild cognitive deficits upon marathon running

Authors: I. LOPEZ[1], S. POZUETA[2], I. YURREBASO-SANTAMARÍA[2], G. GONZALEZ[3], ***C. MATUTE**[3,4,5];
[1]Univ. of Basque Country // CIBERNED // IIS-Biobizkaia, Leioa, Spain; [2]Hosp. Universitario de Cruces, Osakidetza, Barakaldo, Spain; [3]Univ. of the Basque Country, Leioa, Spain; [4]CIBERNED, Leioa, Spain; [5]IIS-Biobizkaia, Leioa, Spain

Abstract: We recently demonstrated that myelin may serve as a source of energy substrates for brain cells during endurance exercise (https://doi.org/10.1038/s42255-025-01244-7). Specifically, we observed a reversible reduction in myelin content within white matter tracts involved in locomotion, motor coordination, somatosensory perception, and emotional processing. In the present study, we investigated whether these myelin changes affect motor and sensory control fidelity, as well as cognitive processing speed. To this end, we conducted a panel of neurophysiological assays to assess nerve conduction velocity in both central and peripheral pathways. We found that the latency of motor, somatosensory, auditory, and visual evoked potentials remained unchanged 24-48 hours after race completion, suggesting that the observed reduction in myelin content does not impair axonal conduction velocity in these pathways. Additionally, we administered neuropsychological tests commonly used in clinical settings to assess cognitive processing speed. Marathon runners performed worse than matched controls on more complex tasks shortly after the race; however, all participants, including controls and runners, achieved similar scores one month later. These findings suggest that marathon runners may experience subtle, transient cognitive deficits that fully resolve within a month. Collectively, our data support the hypothesis that myelin can act as an energy reservoir for neural cells under metabolic stress, while still preserving essential physiological functions and cognitive performance. This integrative approach offers a holistic view of brain adaptation during endurance exercise, revealing functional resilience despite transient structural changes.
Supported by CIBERNED, MCINN, Gobierno Vasco and EITB-Maratoia.

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Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.12/S8

Topic: I.10. Human Learning and Cognition

Support: The study was funded by the Minister of Science under „the Regional Initiative of Excellence Program”.

Title: Assessing the impact of virtual reality technology on reducing stress levels in studying women-preliminary study

Authors: *A. KOZLOWSKA[1], A. SEKOWSKI[2], P. WOJTACHA[3];
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Abstract: Today's sources of stress among young people include changes in the system of living, learning, a passive lifestyle, and the rush of civilization. It is well known that stress causes numerous disorders in the functioning of our body and is a significant risk factor for the development of depression. Moreover, prolonged exposure to stress can also impair memory and cognitive functions, which can affect the ability to learn. Therefore, it is important to look for new methods of supporting students that could allow them to negate the negative effects of chronic stress and thus improve their mental health. Therefore, the purpose of the proposed research is to evaluate the impact of the virtual reality (VR) environment on a group of female students suffering from a high level of stress. The research was conducted in a group of 20 young women aged 20 to 25 years diagnosed with high levels of perceived stress using the Perceived Stress Scale-10 (PSS-10). They participated in 8 sessions (2 sessions per week, 10 minutes each) using a controlled immersive VR environment, which included 1 simulation scenario related to relaxation. To assess the effect of VR on stress reduction (PSS-10) and memory and cognitive functions (Trail Making Test - TMT, Stroop, and 2-back tests) of these subjects, they were tested twice (before and after the VR sessions). In addition, saliva and/or urine samples (in the morning) were secured from all participants in the studied group, before the first and after the last session in VR, to determine cortisol, dehydroepiandrosterone *(*DHEA), serotonin (5-HT), and dopamine (DA) levels by ELISA. Moreover, the following parameters were determined for each participant before and after the VR session: mean arterial pressure (MAP), systolic (SYS), and diastolic blood pressure (DBP), and heart rate (HR). The results showed elevated levels of cortisol, DHEA, and DA in the subjects' saliva or urine, which did not change after a cycle of VR sessions. Urinal levels of 5-HT also did not differ significantly. However, following some VR sessions, changes in circulatory parameters, including a reduction in MAP, SYS, DBP, and HR, were observed. Additionally, more than half of the enrolled (n = 13 out of 20) volunteers reported decreased perceived stress (from high to moderate or low; PSS-10), accompanied by improvements in memory and cognitive function. These findings suggest that VR environments may be an effective, non-pharmacological tool for stress management and cognitive enhancement in young women. The Minister of Science funded the study under „the Regional Initiative of Excellence Program”. Keywords: stress, virtual reality, memory, cortisol, DHEA, serotonin, dopamine

Disclosures: A. Kozlowska: None. **A. Sekowski:** None. **P. Wojtacha:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.13/S9

Topic: G.03. Stress and the Brain

Support: LDRD 25-ERD-015
DE-AC52-07NA27344

Title: Investigating the Gut-Brain Axis in the Susceptible and Resilient Rat to Traumatic Stress Disorder

Authors: M. REISS, A. SEBASTIAN, D. HUMPHREY, L. TRAN, B. GORMAN, N. BE, ***D. LAM**;
Physical Life Sci. Directorate, Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: Traumatic stress (TS) remains a significant challenge to diagnose and treat, affecting up to 30% of active warfighters and veterans. Recent advances suggest that TS is not solely a brain disorder but involves multi-system pathophysiology mediated by the gut-brain axis (GBA)—a complex bi-directional interaction between the brain and gastrointestinal tract. The GBA integrates microbial metabolites, immune system activation, circulatory factors, and vagus nerve signaling, offering a promising avenue for identifying biomarkers and therapeutic targets for TS. Preclinical and clinical studies have demonstrated symptom improvement through gut remodeling using antibiotics, probiotics, or fecal matter transplants, yet the mechanisms by which the GBA responds to traumatic stress remain poorly understood. This study aims to elucidate the role of neuroactive microbial metabolites in GBA-mediated responses to psychological trauma. Using a predator odor rat model, metabolomic profiling will be conducted across three organ systems: the gut, brain, and circulatory system. This approach will identify metabolites shared across these systems, providing insights into the stability and persistence of GBA responses in susceptible versus resilient behavioral phenotypes. By uncovering the metabolic pathways underlying GBA-mediated resilience and susceptibility, this research aims to advance the understanding of TS pathophysiology to inform therapeutic development and strategies.

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Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

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Program #/Poster #: PSTR080.14/S10

Topic: G.03. Stress and the Brain

Support: JSPS Grant JP23H02640

Title: Claustrum ensembles integrate salience and aversive valence signals to facilitate attentional processing

Authors: *A. KASAI[1], Y. YOKOYAMA[2], M. TANUMA[3], H. NOMURA[4], H. HASHIMOTO[5], J. P. JOHANSEN[6];
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Abstract: The ability to detect and respond to salient and negatively valenced stimuli is essential for survival. However, the neural mechanisms that flexibly integrate salience and aversive valence signals depending on internal state remain poorly understood. In this study, we performed in vivo calcium imaging of claustram (CLA) neurons expressing Car12 to examine how their activity reflects behavioral context and emotional state. Using the elevated plus maze and social interaction test, we found that under basal conditions, a subset of CLA^{Car12+} neurons was selectively activated during salience-driven behaviors such as approach, avoidance, and head-dipping. These behaviors are associated with risk assessment and vigilance, suggesting that these neurons contribute to baseline attentional processing. In contrast, after five consecutive days of social defeat stress, a distinct subpopulation of CLA^{Car12+} neurons was newly recruited during SIT. These neurons exhibited enhanced activity in response to social stimuli such as direct interaction with a conspecific and distant gazing. These stimuli, which were previously processed as neutral before stress exposure, appeared to acquire aversive salience following stress. These findings suggest that stress does not alter the response profiles of salience-responsive CLA neurons, but instead leads to the recruitment of a separate subpopulation tuned to aversive salience. The claustrum thus contains functionally heterogeneous ensembles that dynamically encode salience and valence information in a state-dependent manner, supporting context-appropriate attentional and behavioral responses.

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Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.15/S11

Topic: G.03. Stress and the Brain

Support: Korea research foundation grant RS-2024-00440371

Title: Uncontrollable stress impairs the acquisition of conditioned inhibition: implications for the role of the lateral habenula

Authors: *N.-H. KIM[1], J.-S. HAN[2];
[2]Biol. Sci., [1]Konkuk Univ., Seoul, Korea, Republic of

Abstract: Conditioned inhibition, a fundamental behavioral mechanism alongside conditioned excitation, emerges when a conditioned stimulus (CS) becomes associated with the absence of an unconditioned stimulus (US). Previously, we have demonstrated that the lateral habenula (LHb) critically mediates conditioned inhibition through explicitly unpaired training-induced retardation of excitatory conditioning. Uncontrollable stress induces widespread dysregulation in neural systems and learning deficits, with studies linking stress to LHb hyperactivation in both animal models and humans with depression. LHb with stress exposure exhibits impaired processing of reward signals and negative prediction errors, potentially contributing to learning impairments. While LHb integrity is essential for acquiring conditioned inhibition, the impact of stress-induced LHb dysfunction on this process remains unexplored. To address this gap, we investigated the effects of uncontrollable stress on conditioned inhibition. Animals underwent 60 minutes of restraint + 60 tail-shocks, and explicitly unpaired training of a light cue with food was followed 24 hours later. The inhibitory property of light generated by explicitly unpaired training of the light with the food was assessed through a retardation test, where excitatory learning of the light with food was delayed. While unstressed animals showed a significant delay, stressed animals exhibited no such retardation. This finding suggests that uncontrollable stress disrupts the acquisition of conditioned inhibition. Supported by the Korea Research Foundation Grant funded by the Korean Government (RS-2024-00440371 to Jung-Soo Han).

Disclosures: N. Kim: None. **J. Han:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.16/S12

Topic: G.03. Stress and the Brain

Support: NIH Grant: ZIAMH002784

Title: A role for hippocampal adult neurogenesis in coping strategy during an unexpected increase in stress duration

Authors: *S. CAMPBELL, L. WANG, H. A. CAMERON;
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Abstract: Animals employ various coping strategies to mitigate the adverse effects of stress. Active coping involves behaviors aimed at withdrawing from or eliminating stressors, while passive coping is characterized by immobility in response to aversive stimuli. Rodents often transition from active to passive coping when exposed to unavoidable stress. However, prior work has shown that when rats are subjected to a longer-than-expected period of restraint stress, this can disrupt typical habituation, prompting a return to active coping. The hippocampus plays

a crucial role in regulating stress responses and behaviors and is one of the few brain regions that continues to produce new neurons throughout adulthood. Previous studies have shown that this process, known as adult neurogenesis, plays a role in recovery from stress, so we investigated whether adult-born hippocampal neurons also influence coping strategy adaptation during unexpectedly prolonged stress. We used a pharmacogenetic mouse model to inhibit adult hippocampal neurogenesis. Adult male CD-1 mice expressing the herpes simplex virus thymidine kinase (TK) in the neural stem cells were treated with an antiviral drug valganciclovir, which completely suppresses birth of new neurons. After 8 weeks of treatment, TK mice and wild-type (WT) littermate controls underwent a repeated restraint stress (10 min daily, with an unexpected increase to 30-min). We found that TK mice become increasingly passive across days of restraint stress like WT mice but fail to show the increase in active coping seen in WT mice when the duration of stress is increased. Rather, TK mice become even more passive during the unexpected increase in stress duration. Findings from our study suggest that new neurons may help mice shift their behaviors to cope with an unexpected change in stress.

Disclosures: S. Campbell: None. **L. Wang:** None. **H.A. Cameron:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.17/T1

Topic: G.03. Stress and the Brain

Support: DHA Restoral

Title: Acute neurobehavioral effects of repeated diisopropyl fluorophosphate exposure in a rodent model

Authors: *L. OLSEN[1], V. ETHRIDGE[1], J. STRICKER[1], N. GARGAS[2], J. G. ROHAN[2]; [2]Envrn. Hlth. Effects Lab., [1]Naval Med. Res. Unit Dayton, Wright-Patterson AFB, OH

Abstract: Diisopropyl fluorophosphate (DFP) is an irreversible acetylcholinesterase inhibitor with medicinal and toxicological implications. In medicine, DFP is used to treat chronic glaucoma and has potential indications for delaying peripheral neuropathy. As a toxicant, DFP is an organophosphate insecticide that has been used as a surrogate for chemical warfare agent research. Although DFP is a known neurotoxin, acute versus delayed neurologic effects after repeated dosage have yet to be determined. Male Sprague-Dawley rats (n = 18) were given DFP (0.1, 0.5, and 1.0 mg/kg, subcutaneous injection) or vehicle five days/week for four weeks. Blood bio-samples were collected in vivo from the tail vein at baseline and weekly during exposure to confirm acetylcholinesterase (AChE) inhibition. Neurobehavioral assessments were conducted during the week post-DFP exposure conclusion, and after a two-month washout period. Novel Object Recognition (NOR), Passive-avoidance Task (PAT), and Morris-Water Maze (MWM) were used to measure learning and memory while the Elevated Zero Maze (EZM)

was used to assess anxiety-like behavior. Rats were humanely euthanized at both time points for hippocampal electrophysiological analysis. Analysis of blood serum found DFP to successfully induce AChE inhibition. Preliminary results for the acute timepoint found DFP to not influence NOR or MWM performance ($p > 0.05$). However, the highest dose of DFP (1.0 mg/kg) impaired learning in PAT with rats less hesitant to cross than the control group ($p < 0.05$). The 1.0 mg/kg DFP group spent significantly more time in the open arm in EZM compared to the control group ($p < 0.05$), indicating repeated DFP exposure induces an anxiolytic-like effect. Repeated usage or exposure to DFP is sufficient to cause acute impairment in a fear-aggravated learning task and an anxiolytic-like phenotype. Further data analysis of this ongoing study will indicate if these neurobehavioral effects are sustained and if new delayed effects develop, and if these neurobehavioral effects correlate to changes in electrophysiological activity of the CA1 sub-region of the hippocampus. The results of this study provide foundational data for the development of an in silico application to model and predict neurotoxic effects of other pharmacological and neurotoxic compounds. No DoD endorsement implied.

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Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.18/T2

Topic: G.03. Stress and the Brain

Support: R01MH127737A1

Title: Targeting Insulo-frontal pathway to reduce stress-evoked cognitive rigidity

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Abstract: The anterior insular cortex (aIC), a central hub of the salience network, is engaged in cognitive flexibility. Disruption of aIC structure and function is implicated in stress-related mental disorders, often accompanied by cognitive rigidity—a deficit for which effective treatments are lacking. While insular cortex's roles in interoception and emotional regulation are well-studied, little is known about how aIC impairment contributes to cognitive rigidity. Using mouse attentional set-shifting tasks (AST), we identify aIC neurons projecting to the medial prefrontal cortex (mPFC) as key regulators of adaptive decision-making. These neurons show heightened activity following incorrect—but not correct—trials. This elevated activity persists into subsequent trials, providing a salience signal that enhances mPFC activity updates and promotes activity pattern

convergence over trials. Pre-decision optogenetic manipulation of aIC→mPFC projections perturbs mPFC activity updates and impairs AST performance. Moreover, stress abolishes this outcome-dependence of aIC activity and impairs set-shifting. Crucially, selective optogenetic activation of the aIC→mPFC pathway following incorrect trials enhances mPFC activity updates, improves convergence over trials, and restores cognitive flexibility in stressed mice. These findings reveal a previously unrecognized role of the aIC→mPFC circuit in linking trial outcomes to adaptive decision-making and identify this pathway as a promising therapeutic target for addressing stress-induced cognitive rigidity.

Disclosures: S. Ma: None. **K.H. Wang:** None. **Y. Zuo:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.19/T3

Topic: G.03. Stress and the Brain

Title: Motivational Differences Following Yohimbine and Haloperidol After Experiencing Chronic Unpredictable Stress in Male and Female Rats

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Abstract: Major depressive disorder (MDD) is a debilitating disease that impacts a variety of behaviors such as motivation and the ability to experience pleasure. A major risk factor for MDD is chronic stress (Wang et al., 2025), and while both males and females experience stress, females report greater rates of stress and depression (Smith & Wesselbaum, 2025). Exposing rodents to chronic unpredictable stress (CUS) is frequently used to study MDD, as it closely resembles the uncontrollability and unpredictability of stress seen in humans (Sanchez et al., 2025). Chronic stress can produce lasting effects in the brain, including in the noradrenergic (Park, Campbell, & Diamond, 2001) and mesolimbic dopamine systems (Qiao et al., 2020). Yohimbine, an alpha-2 adrenergic receptor antagonist, pharmacologically induces acute stress (Chen et al., 2015), altering reward seeking behaviors (Park, Campbell, & Diamond, 2001), as does haloperidol, a dopamine D2 receptor antagonist, through its effects on dopamine (Errante et al., 2021). The current study tested male and female Sprague Dawley rats' motivation in an operant box using the progressive ratio schedule of reinforcement (PR) for a sucrose pellet after ten days of CUS. To assess whether changes in PR were related to the effort associated with lever pressing, a less palatable food chow was available freely in the operant chamber during testing as sex differences have been observed in effort choice tasks (Errante et al., 2021). Rats were given saline, yohimbine (2 mg/kg; i.p.), and haloperidol (0.1 mg/kg; i.p.) injections separately and counterbalanced following consistent PR responding. Yohimbine increased (p <0.001) correct lever presses in males and females. While there were no significant interactions, effects of stress, or sex, male CUS rats showed a trend towards an increase in correct lever

presses following yohimbine compared to control males ($p = 0.051$). Haloperidol significantly reduced ($p < 0.001$) correct lever presses, however no significant interactions or stress effects were observed. Haloperidol injections also revealed a sex difference ($p = 0.01$) with females having higher lever presses than males. These results are consistent with other research that the acute pharmacological stressor yohimbine increases overall responding for a palatable food reward and suggest a potential sex specific sensitivity to an acute stressor after exposure to chronic stress as well as to haloperidol. Motivational dysfunction is a central symptom of MDD that impacts functioning in many contexts and understanding the contributing neuropharmacology systems may improve future treatment for MDD in both males and females.

Disclosures: S. Muscott: None. **S. Kuhn:** None. **L. Matuszewich:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.20/T4

Topic: G.03. Stress and the Brain

Support: Wellcome-IBRO IW-3169647301

Title: Comparative effects of environmental enrichment and probiotics on depression and memory in a mouse model

Authors: *T. A. TAGOE, A. BANKAH, E. DARKO, E. AMETEFE, K. KUKUIA, B. ARKO-BOHAM;
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Abstract: Depression affects an estimated 5% of the world's population and is predicted to be the leading cause of mortality and morbidity by 2030. Multiple factors increase the susceptibility of depression and its perpetuation in an individual, with diverse pathophysiological mechanisms. This contributes towards the high number of depression cases refractory to available therapeutic interventions, raising the need to explore alternative approaches. Evidence suggests that both environmental enrichment and probiotics may be effective alternatives to treat depression and its associated memory loss. The aim of the study was to compare the effects of both interventions in isolation and as a combination on depression and associated memory impairment. The study involved 42 male ICR mice (6-8 weeks old) split into 6 groups (n = 7 each). The chronic unpredictable mild stress (CUMS) model was used to induce depression in five groups over a 6 week period and the therapeutic effect of environmental enrichment (2 hours daily for 4 weeks) and/or probiotics (10^8 CFU/ml *lactobacillus and Bifidobacterium*) on depression was compared to that of fluoxetine (10 mg/kg daily) via behavioural (Sucrose Preference Test, Open Field Test, Tail Suspension Test, Novel Object Recognition Task, Y-Maze and Morris Water Maze), molecular (BDNF levels) and histological (hippocampal and prefrontal cortex cell counts) techniques. The monotherapy of either environmental enrichment or probiotics improved

depressive symptoms in mice evidenced by promoting healthy weight gain, increasing sucrose preference and reducing anxiety behavior similar to the effects of fluoxetine, with environmental enrichment proving to be more effective. Both monotherapies also improved working and spatial memory as well as neuronal cell count and volume density. The combination therapy did not offer significant benefits in any of the measured parameters, suggesting an absence of synergy in the mechanisms of action between probiotics and environmental enrichment. In conclusion, both probiotics and environmental enrichment ameliorate CUMS-induced depressive symptoms by reducing weight loss, anhedonia, helplessness, neuronal cell loss and anxiety-like behavior, related to depression. The transient periods of environmental enrichment and easy availability of probiotics enhance the clinical relevance of this study and warrants further exploration to elucidate mechanisms of action.

Disclosures: T.A. Tagoe: None. **A. Bankah:** None. **E. Darko:** None. **E. Ametefe:** None. **K. Kukuia:** None. **B. Arko-Boham:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.21/T5

Topic: G.03. Stress and the Brain

Support: Villanova Undergraduate Research Fellowship

Title: Subchronic stress is sufficient to induce behavioral and epigenetic alterations in both male and female mice, but females exhibit increased stress susceptibility

Authors: M. DOMANICO[1], S. STEVENS[2], I. WAINSTON[1], E. KHOO[1], C. MCCALL[1], B. SWACK[2], ***B. D. SACHS**[1];
[1]Psychological and Brain Sci., [2]Villanova Univ., Villanova, PA

Abstract: Stress-related disorders, such as major depression, anxiety disorders, and post-traumatic stress disorder, lead to considerable disease burden worldwide and are notoriously difficult to treat. While the causes of mental illnesses are unknown, many of these disorders are characterized by striking sex differences. Factors ranging from hormone levels to social roles and cultural influences likely contribute to the mental health discrepancies in males and females, and with an improved understanding of how these factors can promote or protect against psychopathology, it may become possible to design interventions that enhance resilience. Preclinical research using rodent models can provide fundamental insight into sex differences in neurobiological consequences of stress, which could aid in this effort and may have important implications for stress-related disorders. Towards this end, the current work evaluated potential sex differences in stress-induced alterations in DNA methylation and behavior in c57BL6 mice. The results indicate that exposure to five days of mild stress induces behavioral dysfunction in the light-dark emergence, open field, and effort-related reward choice tests. Statistical analyses

using two-way ANOVAs revealed that only the effects of stress in the light-dark emergence test were specific to females; both sexes were susceptible to the other behavioral consequences of this stress paradigm. Stress was also shown to increase global DNA methylation in the nucleus accumbens one week following the end of stress exposure in both males and females. However, targeted epigenetic evaluations using methylation-specific PCR revealed sex differences in DNA methylation at sites in the prodynorphin and inhibitory kappa B kinase beta genes in this brain region. Overall, these findings provide further evidence of sex differences in stress susceptibility and suggest that nuanced sex differences in epigenetic adaptations to stress could contribute to the distinct behavioral outcomes in males and females.

Disclosures: M. Domanico: None. **S. Stevens:** None. **I. Wainston:** None. **E. Khoo:** None. **C. McCall:** None. **B. Swack:** None. **B.D. Sachs:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.22/T6

Topic: G.03. Stress and the Brain

Title: Sensitivity to reward and pet ownership as a buffer against mental health consequences after life-stress events

Authors: *E. NARAYAN, L. KAPLOW, F. HEGARTY, C. MIKKELSEN;
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Abstract: Stressful life events are positively associated with increased psychopathology in adolescence. However, previous work has shown that sensitivity to reward and pet ownership buffer against negative mental health consequences from exposure to negative life experiences (Hu et al., 2022; Friedmann et al., 2018). While work has established the benefit of pet ownership on improving mental health through lifestyle improvements, there has been minimal research investigating its neural mechanism. Pet ownership and reward sensitivity are both associated with mental health improvements, and we sought to determine if there was a connection between them. Thus, we investigated four hypotheses using a longitudinal subsample of children enrolled in the Adolescent Brain Cognitive Development (ABCD) study who participated in the Monetary Incentive Delay (MID) task. First, we hypothesized that there would be a positive association between life-stressors and negative psychopathology in our sample. Second, we hypothesized that reward sensitivity, operationalized as increased activity during the anticipation period in the accumbens area for wins versus neutral during the MID task, would moderate the relationship between life-stressors and negative psychopathology. Third, we hypothesized that pet ownership would moderate the relationship between psychopathology and life stressors. Fourth, we hypothesized that pet ownership would moderate the association between life stressors and reward sensitivity. Replicating previous research, we found a significant relationship between life stress and psychopathology in our sample ($p < 0.01$, $\boldsymbol{\beta}$;; =

0.172), measured through scores on the Brief Problem Monitor questionnaire. We also found that reward activity in the left accumbens significantly moderated the relationship between life stress and psychopathology, such that an increase in reward sensitivity decreased the association between life stress and Brief Problem Monitor score. However, we found no moderating effect of reward activity in the right accumbens (p = 0.07, **β**;; = 0.188). Pet ownership was not a significant moderator of the relationship between life stress and Brief Problem Monitor score (p = 0.925, **β**;; = 0.170), or the relationship between life stress and reward sensitivity for either the left (p = 0.925, **β**;; = -9.74*10-5) or right (p = 0.925, **β**;; = 0.057) accumbens. These findings are critical for promoting an understanding of the important role of reward processing in modulating resilience. Additionally, this research encourages researchers to look for other mechanisms underlying the supportive psychological effects of pet ownership.

Disclosures: E. Narayan: None. **L. Kaplow:** None. **F. Hegarty:** None. **C. Mikkelsen:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.23/T7

Topic: G.03. Stress and the Brain

Support: SECIHTI CF-2023-I-355

Title: Effect of stress and palatable food intake on corticosterone levels and on activation of MC4R neurons in the hypothalamic paraventricular nucleus

Authors: *E. ESPITIA, -Bautista[1], C. GARCIA-LUNA[2], P. SOBERANES-CHAVEZ[3], E. ALVAREZ[4], P. DE GORTARI[5];
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Abstract: Obesity is a global health concern linked to the excessive intake of high-fat, high-sugar foods, often combined with sedentary and stressful lifestyles. Chronic stress exposure affects feeding behavior, promoting palatable food (PF) preference and impairing satiety mechanisms. This study investigates the impact of chronic isolation stress (I-S) and of PF intake on the activation of melanocortin-4 receptor (MC4R)-expressing neurons in the anterior and medial paraventricular hypothalamus (aPVN, mPVN), a region implicated in satiety regulation. We registered daily chow and PF intake as well as weekly body weight. After sacrificed, their intracardial perfused brains obtained and using immunofluorescence we assessed MC4R+c-Fos expression in the aPVN and mPVN under different feeding and stress conditions. Forty male rats (250 g) were divided into two dietary groups: chow (n=20) or chocolate milk PF (n=20) and

either exposed to I-S or not for two weeks, resulting 4 groups. Following a 48-hour fasting period, 8 rats were euthanized; the remaining were re-fed with either chow (n=16) or PF (n=16) for 2 h before euthanasia to evaluate whether the refeeding food quality is important for activation of the satiety neural circuit Our results showed that PF-fed animals consumed more kcal than chow-fed, regardless the stress exposure (60% more) and showed lower corticosterone levels (20% less) than chow stressed =100%. In the aPVN, PF intake (stressed or not) reduced activation of MC4R neurons under both fasting and ad libitum conditions Notably, rats previously fed PF and re-fed with chow also showed decreased MC4R activation, while those re-fed with PF had the lowest levels of activation (35% less), which correlated with their overconsumption behavior. In mPVN, differences in MC4R activation were observed only under ad libitum conditions, where I-S PF-fed rats showed reduced neuronal activation 80% less than control rats. These findings suggest that the combination of chronic stress and PF consumption disrupts MC4R signaling in the PVN, contributing to impaired satiety and excessive caloric intake. Our previous data from this model also showed alterations in dopamine and serotonin levels in the nucleus accumbens, indicating a potential link between hypothalamic MC4R signaling and reward-related circuits in stress-induced overeating. This work was supported by SECIHTI CF-2023-I-355 (PG) and CONAHCyT postdoctoral fellowship (to EEB; CVU: 559045).

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Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.24/T8

Topic: G.03. Stress and the Brain

Support: DGAPA-PAPIIT IN 225925
PAPIME PE 305 125

Title: Behavioral responses in social isolated pregnant rats

Authors: *A. C. HERNÁNDEZ SÁNCHEZ[1], P. TORRES-CARRILLO[2], E. M. ALARCON[3], D. B. PAZ-TREJO[4], H. SANCHEZ-CASTILLO[5];
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Abstract: Previous research on the gestational process has been established it as a period in which several cognitive and behavioral changes happened. Those changes can be influenced by stressful situations. Particularly, the COVID-19 pandemic brought special attention to the effects

of social isolation in different sex, ages, etc. This stressful situations could result in a predisposition to develop stress-related disorders like depression, anxiety, trauma, etc. In this context, social isolation during pregnancy may have effects over the whole process of pregnancy; however, there is not yet enough research to support such a claim. The main objective of this study was to explore the effects of social isolation during pregnancy in wistar rats. Female pregnant rats under conditions of social isolation and non-isolation were evaluated in the forced swim test (FST), open field test (OFT), and Barnes maze (BM). The results revealed that the isolated group showed a higher number of errors in the BM, along with a tendency for long latencies to reach the escape hole, suggesting potential memory impairment. In the OFT, isolated rats presented a tendency to make fewer crosses trough the center, which has been described as an anxiety-like behavior. Finally, in the FST, the isolated group showed more immobility and less climbing time, but, this results were non-significant.These results allow us to studying the potential cumulative effects of the interaction between social isolation and the pregnancy period. That could mean that social isolation may increased anxiety and depression-like behaviors, as well as memory impairment. It is important to consider the effects of stressful situations on the pregnant population, such as the COVID-19 pandemic and the social isolation that the world faced.

Disclosures: A.C. Hernández Sánchez: None. **P. Torres-Carrillo:** None. **E.M. Alarcon:** None. **D.B. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.25/T9

Topic: G.03. Stress and the Brain

Support: DGAPA-PAPIIT IN 225 925
DGAPA-PAPIME PE 305 125

Title: The effect of environmental enrichment on females and males exposed to chronic stress

Authors: *P. TORRES-CARRILLO[1], A. C. HERNÁNDEZ SÁNCHEZ[1], E. ALARCON[2], D. B. PAZ-TREJO[3], H. SANCHEZ-CASTILLO[4];
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Abstract: Chronic stress exposure allows the emergence of depression-like and anxiety-like behaviors, as well as cognitive impairments, particularly those related to learning and memory. Besides a common problem in stress studies, is not included females despite of the risk of developing a stress-related disorders are higher in women than in men. Besides, environmental conditions such as housing and husbandry have a major impact on the laboratory animal

throughout its life and will thereby influence the results of the animal experimentation. On the other hand, the usual care and maintenance of laboratory animals does not always include species specific needs in relation to their environment. Environmental enrichment (EE) has been defined as a measure that promotes the expression of species-specific natural behaviors and, at the same time, may reduce abnormal behaviors. Moreover, several studies have demonstrated the improvement effects of environmental enrichment in subjects exposed to stress. The main goal was to evaluate the effects of EE on chronic unpredictable stress conditions. Female and male Wistar rats (12 weeks old) were used. The stressor used was the Chronic Unpredictable Stress Battery (CUSB), which consists of six stressors applied randomly over a period of 10 days. Environmental enrichment (EE) consisted of paper rolls that could be used as shelters or for gnawing, and access to foods such as amaranth, blueberries, sunflower seeds, and marshmallows 3 times per day for all experiments. Subjects were assigned to the following groups: control, CUSB, EE, and CUSB+EE. All subjects were evaluated with the elevated zero maze test (EZM), novel object recognition test (NOR) and saccharine preference test (SP). The results obtained in EZM and NOR showed that the CUSB+EE group increased both the number of entries, and the time spent in the open arms, as well as the time spent exploring the novel object, compared to the CUSB group. An increase in risk assessment behaviors was also observed in the EE group in the zero maze, indicating that the change in environment enhanced exploratory behavior in the subjects. No effects were observed in the saccharin preference test. These effects were consistently observed in both males and females. Despite of the positive results found in this study, it is necessary to standardize the elements used in EE studies, as well as to investigate the neurobiological mechanisms underlying the improvement effects of EE under stress conditions.

Disclosures: P. Torres-Carrillo: None. **A.C. Hernández Sánchez:** None. **E. Alarcon:** None. **D.B. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.26/T10

Topic: G.03. Stress and the Brain

Support: DGAPA-PAPIIT IN 225 925
PAPIME PE 305 125

Title: Glucocorticoid receptors in Octopus maya nervous system

Authors: *F. SÁNCHEZ-FLORES[1], N. TRUJILLO GUTIÉRREZ[1], J. F. VERGARA-OVALLE[3], O. R. GALICIA, Sr.[4], D. B. PAZ-TREJO[1], H. SANCHEZ-CASTILLO[2]; [2]Psychobiology and Neurosciences, [1]Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [3]Biochem., UNAM, Cd de Mexico, Mexico; [4]Univ. Iberoamericana, Lomas de Santa Fe, Mexico

Abstract: Stress is defined as a response to any perceived or imminent harmful stimuli, it´s a widely extended behavior present in both vertebrate and invertebrate phyla. In mammals, this response is composed of multiple physiological variables whose interactions create a series of biological circuits that constantly send feedback information, however, in invertebrates, particularly octopuses, is not well studied. One of the most common components of stress response among vertebrates is the glucocorticoid system, which is involved with the immune, metabolic, cognitive effects, etc. It´s well known that the Octopuses show a complex behavioral repertoire and are capable of solve learning and memory tasks, representing a unique model with totally different nervous organization, but there is not much information about the presence of glucocorticoids in cephalopods, such as cortisol. The identification and localization of the glucocorticoid receptors in the octopuses´ nervous system is a major step in the description of its stress mechanisms. We used immunofluorescence as our tool to identify the distribution of glucocorticoid receptors along the *Octopus maya* nervous system with an emphasis in the central ganglion. We identify the presence of glucocorticoid receptors, but the fluorescence index is not sufficient to get significant differences between octopus-lobes. Further quantitative methods will be needed.

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Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.27/T11

Topic: G.03. Stress and the Brain

Title: Enhancing Attentional Resource Efficiency via Microcone Patch Stimulation: NIRS-based Evidence from Functional Dynamics and Network Interactions

Authors: *A. KAWASAKI;
Ritsumeikan Univ., Kyoto-Shi, Japan

Abstract: Recent interest in non-invasive neuromodulation has explored the role of auricular stimulation in optimizing cognitive processes. Here, we investigated the effects of a wearable microcone patch stimulator on neurocognitive efficiency using multi-channel near-infrared spectroscopy (NIRS). A total of 15-channel oxy-Hb signals were recorded during rest and task phases under two conditions (patch vs. control) in a within-subjects design. We focused on three composite indices derived from spatially defined channels: DMN (CH3+CH15), FTN/SN (CH6+CH14), and WMN (CH1).
Linear mixed model (LMM) analyses revealed significant interaction effects between stimulation and task for DMN [Task × Patch: $p = .043$], showing that DMN activity was significantly reduced during task under microcone patch stimulation, suggesting effective suppression of internally oriented processing. The FTN/SN index showed a significant main effect of Patch [$p =$

.022], indicating global downregulation of cognitive monitoring irrespective of task state. CH1 (WMN) activity tended to decrease during task in the patch group ($p = .072$), without compromising task performance, suggesting enhanced efficiency in working memory-related processing.
Behavioral performance (accuracy) did not differ between conditions ($p > .3$), reinforcing the interpretation of resource optimization rather than mere activation suppression. Network analysis further revealed that functional connectivity between WMN (CH1) and FTN/SN (CH6/CH14) significantly decreased under patch stimulation [$r = .62$ vs. $.38$, $z = 2.07$, $p < .05$], while intra-network correlation between CH6 and CH14 increased, indicating preserved or enhanced synchrony within the monitoring network.
These findings suggest that microcone patch stimulation promotes functional segregation of high-load networks, reduces redundant monitoring, and facilitates dynamic switching — contributing to attentional efficiency. As such, the method may serve as a low-burden neuromodulatory tool for cognitive enhancement across populations. All procedures were approved by the institutional ethics committee, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Disclosures: A. Kawasaki: None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.28/Web Only

Topic: G.03. Stress and the Brain

Title: Mental fatigue through alpha-theta-beta detection in drivers based on EEG signals using machine learning algorithms based on fractal analysis

Authors: M. RAMÍREZ[1], ***M. L. GUTIÉRREZ-MUÑOZ**[2], X. ORTIZ[3], D. MARTINEZ-PEON[1];
[1]Dep. of Electrical and Electronics Eng.– Postgraduate Studies Div., Tecnológico Nacional de México – IT Nuevo León, Guadalupe, Mexico; [3]Lab. de Estudios Multidisciplinarios de la Conducta, [2]Univ. Autonoma de Nuevo Leon, Facultad de Psicologia, Monterrey, Mexico

Abstract: Mental fatigue is a condition that reduces cognitive performance and compromises safety during driving. Its timely detection is essential to prevent traffic accidents related to attentional lapses. This study proposes a detection approach based on electroencephalographic (EEG) signals and machine learning algorithms based on fractal analysis to identify mental fatigue states in drivers. The experiment was conducted with 4 participants (3 men and 1 women) aged between 20 and 30 years, all with prior driving experience. As an initial phase, a three-minute baseline test was performed in which the subjects fixed on a static point on a screen, both with eyes open and closed, to confirm that they were in an alert state without signs of fatigue. EEG signals from this baseline were used to extract frequency bands—alpha, theta, and beta—

for analysis. Subsequently, participants performed a continuous driving task for 60 minutes. The EEG signals recorded during this stage underwent preprocessing, including data normalization, downsampling, and segmentation by specific frequency bands for comparison with the baseline. To evaluate changes associated with fatigue progression, the Event-Related Desynchronization/Synchronization (ERD/ERS) technique was applied, allowing the quantification of power variations in EEG bands between the baseline condition and different moments during driving. In addition, fractal-based algorithms—such as the Hurst exponent and Higuchi's fractal dimension—were used as input features for machine learning models designed to classify mental states. Repeated-measures ANOVA tests were applied to statistically analyze the differences across experimental stages, revealing significant differences in the theta and alpha bands between the beginning and intermediate driving phases ($p < 0.05$). Furthermore, several supervised classification algorithms—including support vector machines (SVM), random forests, and neural networks—were evaluated, achieving accuracies above 75% in detecting fatigue states. These findings confirm the viability of the proposed approach and its potential application in intelligent systems for real-time cognitive monitoring of drivers.

Disclosures: M. Ramírez: None. **M.L. Gutiérrez-Muñoz:** None. **X. Ortiz:** None. **D. Martinez-Peon:** None.

Poster

PSTR080: Stress Impacts on Cognition, Memory, and Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR080.29/T12

Topic: G.03. Stress and the Brain

Title: Limitations of current regulatory frameworks to assess the potential hazards of high frequency electromagnetic radiation

Authors: *A. C. BASU[1], S. GOVINDASAMY[2], M. S. DELATTE[3], K. FRANCO[1];
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Abstract: Most commercially available wireless communication devices utilize electromagnetic radiation at gigahertz frequencies for data transmission. As use of the gigahertz range is increasing, expansion into the tens or hundreds of gigahertz, and even the terahertz range is imminent. How might increased use of high frequency electromagnetic signals affect brain function in the future, and what hazard assessment approach should be used to understand potential risks to human and animal health? We propose an approach that draws upon established hazard assessment principles, employs realistic modeling of electromagnetic radiation exposure patterns (in terms of frequency, duration, burstiness, and intensity), and examines systems and behavioral neuroscience endpoints. The federal agencies that have historically been responsible for hazard assessment related to electromagnetic radiation include the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the Federal Communications Commission (FCC). Currently, they focus on high intensity and short duration exposure and the

measures they routinely assess in these studies focus on DNA damage and cellular toxicity. We propose the expansion of existing hazard assessment paradigms for electromagnetic radiation to consider the effects of low intensity, long duration exposure on cellular function, circuits, systems, behavior, and cognition, with a primary focus on hippocampus-dependent functions due to their conservation across rodents and humans.

Disclosures: A.C. Basu: None. **S. Govindasamy:** None. **M.S. Delatte:** None. **K. Franco:** None.

Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.01/T13

Topic: G.06. Autonomic Regulation

Title: The body speaks, physiological markers of social anxiety

Authors: *L. DE FRANCESCO[1], O. DAL MONTE[2];
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Abstract: Successful social interactions rely on monitoring behavioral cues like interpersonal distance and emotional expressions. Although these factors have been widely studied, it remains challenging to understand their impact on psychophysiology in realistic controlled environments, or how social anxiety shapes these responses. To examine how individuals respond to complex social scenarios, we systematically manipulated interpersonal distance, avatar emotional expressions, and eye contact within a 3D virtual environment. Electrodermal activity (EDA) increased as avatars approached participants, and this physiological response was further modulated by the emotional expressions of nearby avatars. Notably, subjective reports of social discomfort closely aligned with these implicit physiological measures. Participants reported greater discomfort in scenarios where avatars displayed fearful or angry expressions at close proximity. Furthermore, individuals with high versus low social anxiety exhibited distinct patterns of physiological and subjective responses, which were also shaped by atypical exploratory behaviors directed toward the avatars. Our findings show how the interplay of multiple factors within complex virtual social environments shapes psycho-physiological responses, with these effects further modulated by the level of social anxiety. These results offer valuable insights for the development of innovative, immersive training programs aimed at enhancing social skills.

Disclosures: L. De Francesco: None. **O. Dal Monte:** None.

Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.02/T14

Topic: G.06. Autonomic Regulation

Support: Grant-in-Aid for Scientific Research (B) (23H02137)

Title: Molecularly defined neurons in anterodorsal preoptic nucleus counterbalance torpor

Authors: *A. OHBA[1,2], N. FUKATSU[1,3], D. ONO[5], A. YAMANAKA[6], H. WAKE[4,1,7,8], H. YAMAGUCHI[1];
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Abstract: When faced with food scarcity, some animals survive by entering hypometabolic and hypothermic states referred to as torpor. Torpor is intermittently interrupted by periods of euthermia, known as inter-bout arousal (IBA). The balance between heat production and its suppression must be tightly regulated since failure to rewarm from torpor can be fatal. Although recent works have uncovered the neural circuits that induce torpor in mice by suppressing metabolism and thermogenesis, little is known about the pathways that drive heat production during torpor to enable successful arousal. To identify the neural circuits that counteract torpor, we performed a brain-wide mapping of phospho-S6 (pS6) signals in fasted mice at an ambient temperature of 16 ℃, collecting the brain during the rewarming phase. Brains were subsequently subjected to immunostaining with anti-pS6 antibodies, cleared using iDISCO, imaged with light-sheet microscopy, and analyzed using ClearMap. Compared with ad libitum-fed control mice harvested at the same time point, we observed selective activation of neurons in the anterodorsal preoptic nucleus (ADP), a small, understudied nucleus in the dorsal part of the preoptic area. Immunohistochemistry further revealed that ADP neurons express corticotropin-releasing factor (CRF), distinguishing them from neighboring cells in the ventral BNST and establishing CRF as a region-specific marker. To examine the functional role of ADP-CRF neurons in torpor regulation, we chemogenetically inhibited or ablated these neurons. Both manipulations deepened and prolonged torpor bouts, indicating that ADP-CRF neuron activity is indispensable for preventing excessive hypothermia during torpor. We then hypothesized that activating ADP-CRF neurons could block torpor entry in response to energy deficits. Using closed-loop optogenetic stimulation triggered by a surface temperature drop below 33 ℃, we found that activation of ADP-CRF neurons entirely prevented torpor onset. Finally, we assessed whether ADP-CRF neurons drive thermogenesis in brown adipose tissue (BAT) under ad libitum feeding. Optogenetic stimulation elicited a significant increase in both BAT temperature and locomotor activity, confirming their thermogenic function. Overall, these findings demonstrate that ADP-CRF neurons counteract torpor by promoting heat production, offering new insights into the neural control of hypometabolism and arousal.

Disclosures: A. Ohba: None. **N. Fukatsu:** None. **D. Ono:** None. **A. Yamanaka:** None. **H. Wake:** None. **H. Yamaguchi:** None.

Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.03/T15

Topic: G.06. Autonomic Regulation

Title: Electrophysiological properties and temperature sensitivities of hypothalamic neurons in a hibernating and a non-hibernating mammal

Authors: *C. SILVA RUBIO[1], F. VAN BREUKELEN[2], D. H. BRAGER[2];
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Abstract: Thermoregulation is essential for survival in all mammals. Environmental temperature must therefore be continuously monitored by the nervous system to produce appropriate behavioral and physiological responses. A combination of feedforward and feedback mechanisms converge on the medial preoptic area of the hypothalamus (mPOA) to regulate core body temperature. These thermoregulatory circuits were primarily studied in mice and rats, which maintain a relatively high and stable core body temperature at 37°C across a specified range of environmental temperatures. Ground squirrels by contrast are mammals that lower their core body temperature due to a depression in their metabolic rate throughout the winter season. It is unknown whether the electrophysiological properties and temperature sensitivities of mPOA neurons are similar across these mammals that differ in their thermoregulatory strategies. We used current clamp recordings to measure the intrinsic and action potential firing properties of mPOA neurons in acute brain slices from mice and golden-mantled ground squirrels (*Callospermophilus lateralis*). We found no differences in resting membrane potential, input resistance, and membrane time constant across species. However, both spontaneous and driven action potential firing was higher in mouse mPOA neurons compared to the ground squirrel. Experiments to determine the biophysical mechanisms for this difference and whether temperature sensitivities of mPOA neurons would differ between these two species are still under continuation. These experiments will provide a framework to investigate the neural basis of thermoregulation across hibernating and non-hibernating species.

Disclosures: C. Silva Rubio: None. **F. van Breukelen:** None. **D.H. Brager:** None.

Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.04/T16

Topic: G.06. Autonomic Regulation

Support: DP1DK 143574

Title: Ultrasound stimulation of the hypothalamus evokes a hypothermia and hypometabolism state through activation of functionally and molecularly distinct neurons

Authors: *D. ZHANG[1,2], L. YANG[1], Y. YUE[1], J. YUAN[1], Y. YANG[1], H. CHEN[1,2];
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Abstract: Ultrasound stimulation of the hypothalamic preoptic area (POA) can noninvasively evoke a torpor-like hypothermia and hypometabolism state in mice (Yang et al., 2023). We hypothesized that this effect arises from ultrasound activation of torpor-regulating neurons within the POA. To test this hypothesis, we developed a lightweight (≤2.2 g) head-mounted acousto-optic device that enables simultaneous single-neuron calcium imaging and focused ultrasound stimulation in awake, freely moving mice. The device integrates a miniaturized one-photon fluorescence microscope (miniscope, Inscopix) with a GRIN lens for single-neuron recording from a large POA neuronal population, and a wearable ultrasound transducer confocal with the miniscope optical axis. During experiments, mice moved freely in their home cage while behavioral responses were recorded via thermal and video cameras; metabolic rate was assessed in a separate chamber using indirect calorimetry. Female C57BL/6J mice (8-10 weeks old, n = 30) were injected with AAV5-SYN-GCaMP6s in the POA and implanted with a GRIN lens. After recovery, we delivered ultrasound stimulation at two pressure levels, 1 MPa and 1.5 MPa to the POA and monitored interscapular brown adipose tissue (BAT) temperature and oxygen consumption (VO_2). Ultrasound stimulation at 1.5 MPa induced a pronounced decrease in BAT temperature (ΔT: -2.9 ± 0.4 °C, $p < 0.01$) and VO_2 (-33.8 ± 5.2 %, $p < 0.05$), whereas ultrasound stimulation at 1 MPa produced a moderate but significant suppression (ΔT: -1.6 ± 0.3 °C, $p < 0.01$; VO_2: -19.7 ± 3.8 %, $p < 0.05$). In vivo calcium imaging revealed three principal response types, nonresponsive, activated, and inhibited neurons. To identify molecular signatures of ultrasound-activated POA neurons, we performed single-nucleus RNA sequencing (snRNA-seq) on POA tissue harvested immediately after stimulation. Clustering analysis (Seurat) defined 38 distinct cell types; 14 clusters showed significant upregulation of immediate early genes (IEGs: Fos, Egr1, Nr4a1, etc). Among these, one cluster expressed known torpor-regulating markers (Adcyap1, Bdnf, Esr1). Differential gene expression within this cluster revealed overexpression of mechanosensitive channels (Kcnk2, Trpc4), suggesting that FUS engages torpor-regulating neurons via activation of mechanosenstivie ion channels. Collectively, these findings establish a molecularly distinct set of POA neurons engaged by FUS and elucidate the molecular and cellular mechanisms underlying ultrasound-induced thermometabolic modulation.

Disclosures: D. Zhang: None. **L. Yang:** None. **Y. Yue:** None. **J. Yuan:** None. **Y. Yang:** None. **H. Chen:** None.

Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.05/U1

Topic: G.06. Autonomic Regulation

Support: Ray Gaskins Health Sciences Graduate Fellowship

Title: The neural correlates of therapeutic interventions rooted in breathing manipulations

Authors: *N. RAHEJA[1], L. P. SANDS, III[2], U. KIRK[5], A. BINKS[3], J. P. WHITE[4], V. JATLA[3], J. WONG[6], L. BARBOSA[3], T. M. LOHRENZ[3], P. MONTAGUE[7];
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Abstract: Slow-paced breathing has emerged as a prominent intervention to reduce anxiety and stress in both clinical and everyday settings. These practices center around shifting focus inwards to cultivate awareness of our bodily sensations to consciously control our breathing rhythm. Despite their widespread use, the simultaneous neural correlates of these techniques remain unknown. In this research, we employed a novel method, intracranial electrochemistry, in seizure disorder patients in the epilepsy monitoring unit to allow for direct sub-second recordings of dopamine, serotonin, norepinephrine, and pH. This method relies on deep learning neural networks to perform inference on neurochemical concentrations of these monoamines. These recordings, paired with simultaneous nasal respiration monitoring, provide the first conscious human dataset capturing monoaminergic fluctuations aligned with structured breathing patterns across multiple brain regions. During these recordings, participants engaged in a slow-paced breathing exercise known as box breathing. Here we demonstrate a unique neural signature of deliberate, structured breathing specific to the amygdala. We first identified a region-specific respiratory-driven pattern difference during spontaneous, resting breathing and slow-paced breathing. During both breathing patterns, there is oscillation coordination between serotonin (5HT), norepinephrine (NE), and pH signals that is uniquely localized to the amygdala. Box breathing induced a slower, more temporally organized oscillatory rhythm and reduced, yet structured fluctuation of neurotransmitter signals. Although some oscillatory fluctuations are observed, these same patterns of neuromodulator coordination in the amygdala did not emerge in the anterior cingulate cortex (ACC). These data indicate that conscious breath control can act as a neural metronome, directly coordinating neuromodulatory fluctuations in brain regions involved in affective processing, offering a neurobiological basis for its therapeutic effects and widespread use.

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Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.06/U2

Topic: G.06. Autonomic Regulation

Title: Forced walking induces a consistent brain and body temperature drop and modulates brain activity in mice

Authors: T. YI[1], ***O. YAGHMAZADEH**[4,2], G. BUZSAKI[3];
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Abstract: Behavioral experiments are a cornerstone of modern neuroscience. In particular, walking and running mice have served as a versatile model, widely used to investigate motor control, sensory integration, and decision-making, as well as in research on neurological disorders. Understanding how physical activity influences brain function is crucial for advancing our comprehension of brain-body interactions and for elucidating the mechanisms by which such activities impact and can be leveraged to improve mental health outcomes. Here, we report an unexpected observation of a steady body and brain temperature drop in mice subjected to forced walking, along with the resulting changes in their neural activity. Adult C57BL/6J mice were trained on a speed-controlled treadmill (5 cm/s) or a voluntary wheel (up to 70 cm/s) for up to 40 minutes. Chronically implanted thermocouples monitored brain temperature before, during, and after running. Neuropixel 1.0 probes recorded activity from single neurons across the hippocampus, dorsal striatum, thalamus, and motor cortex regions. Putative pyramidal cells, interneurons, and medium spiny neurons were classified based on waveform properties. A significant drop in body and brain temperature (up to 3°C) was consistently observed in mice performing forced walking, but not in those running voluntarily in a wheel. Our electrophysiological results indicate that neural activity was significantly affected through this behavioral task in specific brain region-cell type combinations indicating that the temperature drop influenced the ongoing brain activity. Overall, our findings demonstrate that inducing physiological changes through behavioral tasks can substantially modulate neural activity, partially by changing brain temperature. This suggests that such physiologically driven alterations could be leveraged to induce or enhance neuromodulatory effects that can be potentially employed in the treatment of various brain disorders.

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Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

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Program #/Poster #: PSTR081.07/U3

Topic: G.06. Autonomic Regulation

Support: NIH HL166183
MU REP Postdoctoral Fellowship awarded to HRW

Title: Sustained hypoxia exposure increases respiration and nucleus tractus solitarii (nTS) activity due to differential effects on GABAergic and non-GABAergic nTS neurons

Authors: *H. R. WRIGHT[1,2], G. C. HOFMANN[2], H. A. DANTZLER[2], D. D. KLINE[2]; [1]Univ. of Missouri, Columbia, MO; [2]Biomed. Sciences/Dalton CRC, Univ. of Missouri, Columbia, Columbia, MO

Abstract: Exposure to hypoxia occurs during physiological (e.g., high altitudes) and pathophysiological conditions (e.g., obstructive pulmonary disease). The nucleus tractus solitarii (nTS) integrates sensory input from peripheral chemoreceptors. nTS activity is determined through balanced glutamate (Glu) and gamma-aminobutyric acid (GABA) signaling, which is critical for generating proper reflexes and maintaining cardiorespiratory homeostasis in response to hypoxia. This study investigated how sustained hypoxia (SH) affects GABA neurons in the nTS. We hypothesized that reduced GABAergic inhibition increases nTS excitability and cardiorespiratory function following hypoxia. Male and female GAD1-EGFP mice [strain FVB-Tg (GadGFP) 45704Swn/J, Jackson Laboratory, 4-12 weeks] were used to differentiate GABA (GAD+) and presumptive Glu (GAD-) neurons in the nTS. Mice were exposed to sustained hypoxia (SH, 10% O2, 24 hours) or normoxia (21% O2, 24 hours) before experimentation. First, plethysmography was used to monitor respiratory parameters in mice before and after SH. We observed increased tidal volume and minute ventilation following SH exposure. Next, immunohistochemistry (IHC) was used to identify recently activated GAD+ or GAD- neurons in the nTS by staining for c-Fos, which is increased in the nTS after SH. Similarly, SH exposure increases overall neuronal activity within the nTS as measured by extracellular multi-electrode array (MEA) recordings of spontaneous action potentials (APs) in horizontal brainstem slices. Next, whole-cell patch-clamp recordings were used to examine synaptic neurotransmission and electrophysiological properties of GAD+ and GAD- nTS neurons. Neurons were further characterized by mono- or polysynaptic connection with the tractus solitarii (TS) via TS stimulation. We report that SH increases the frequency of spontaneous excitatory post synaptic currents onto both mono- and polysynaptic GAD+ neurons and increases synaptic throughput of polysynaptic GAD+ neurons at 20hz TS stimulation. To determine if SH alters tonic GABA regulation within the nTS, we applied the $GABA_A$ receptor antagonist gabazine (GBZ, 25uM). We found that GBZ application increases AP count at 20hz TS stimulation in both monosynaptic GAD+ and GAD- neurons from normoxic controls, but has no effect on monosynaptic GAD- neurons following SH. Similarly, application of GBZ during MEA recordings had a greater effect on the activity of nTS neurons from normoxic mice, compared to SH. Taken together, these data indicate an SH-induced increase in the activity of non-GABAergic NTS neurons likely via alterations to GABA influence on local NTS circuitry.

Disclosures: H.R. Wright: None. **G.C. Hofmann:** None. **H.A. Dantzler:** None. **D.D. Kline:** None.

Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.08/U4

Topic: G.06. Autonomic Regulation

Support: T32 DK112751

Title: Molecular and spatial characterization of caudal raphe cell types mediating thermogenesis.

Authors: *B. MARTIN[1], J. M. RESCH[1], M. MCDONOUGH[1], T. C. BUTLER[3], A. BERNS[2]; [1]Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA; [2]Univ. of Iowa, Iowa City, IA, ; [3]The Univ. Of Iowa Neurosci. Grad. Program, Iowa City, IA

Abstract: The caudal raphe (CR), which encompasses the raphe magnus (RMg), raphe pallidus (RPa), raphe obscurus (ROb), and the parapyramidal region, is critical for thermoregulation. Specifically, the RPa is a major premotor site for regulating sympathetic outflow to thermogenic fat. Vesicular glutamate transporter type 3 (*Slc17a8;* VGLUT3)-expressing RPa neurons markedly project to sympathetic preganglionic neurons in the spinal cord and their chemogenetic activation increases brown adipose tissue (BAT) thermogenesis. That said, RPa^{VGLUT3} neurons also project to the lateral parabrachial nucleus, nucleus ambiguus, ventrolateral medulla, and dorsal motor nucleus of the vagus. Given the diversity of projections, there is likely functional heterogeneity amongst different RPa^{VGLUT3} neuron subtypes. While prior single-cell transcriptomic studies have comprehensively characterized the dorsal and median raphe regions there is a lack of similarly detailed analysis of the CR. To further investigate the CR and genetically define different RPa^{VGLUT3} neuronal populations, we performed single-nucleus RNA sequencing (snRNA-seq) with an enrichment of RPa^{VGLUT3} neurons. Our sequencing results identified glutamatergic, GABAergic, and serotonergic neuronal subtypes, 11 of which were transcriptionally distinct neuron populations expressing VGLUT3 and/or serotonin markers—which we further characterized spatially with multiplexed fluorescence *in situ* hybridization. Importantly, our transcriptomic analysis identified thyrotropin-releasing hormone (*Trh*)-expressing neurons in the RPa, which also express VGLUT3 and have been previously shown to be activated by cold. Accordingly, we show that chemogenetic activation of RPa^{Trh} neurons drives increased BAT thermogenesis comparable to activation of RPa^{VGLUT3} neurons. Additionally, activation of RPa^{Trh} neurons drives increased oxygen consumption and core body temperature without altering locomotor activity. Future work will fully characterize newly-defined CR neuronal populations critical for thermoregulation and energy homeostasis.

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Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR081.09/U5

Topic: G.06. Autonomic Regulation

Support: NSF Grant FAIN#2440777

Title: The duration of light exposure differentially affects tidal volume and respiratory frequency in male mice.

Authors: *G. M. MARINO[1], R. C. TWINING[2], D. M. ARBLE[1];
[1]Dept. of Biol. Sci., [2]Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Respiratory frequency and tidal volume exhibit daily, 24-hr rhythms in human and rodent models. Environmental light has emerged as a potential modulator of ventilatory rhythmicity, as mice lacking intrinsically photosensitive retinal ganglion cells fail to alter their breathing in response to light. Despite this evidence, it remains unknown how the duration of light exposure influences breathing in mice. To assess the effects of light exposure on breathing, male wild-type mice (n = 8) were exposed to broad-spectrum white light (~450 lux) in the standard dark phase, either for 3h (ZT 13-16) or for 5 minutes (ZT13-13.05). Respiratory measures were assessed for 36 hours using whole-body plethysmography. To determine whether the light manipulation produced significant deviations from expected respiratory patterns, a nonparametric, within-subjects bootstrapping approach was conducted in R. This compared parallel time points between a predefined test period and the equivalent control period without a light manipulation. We found that a 3h light stimulus administered during the standard dark phase reduced tidal volume and respiratory frequency for ~80 minutes during light exposure. Immediately following the offset of the 3h stimulus, respiratory frequency was increased for 2h compared to control. While statistical analysis is currently ongoing, a 5-minute light stimulus appeared to decrease both tidal volume and respiratory frequency during light exposure. In contrast to a 3hr stimulus, both tidal volume and respiratory frequency were increased at 90 minutes following the offset of the 5-minute light stimulus. While preliminary, these data suggest that tidal volume and respiratory frequency differentially respond to the duration of environmental light exposure. Subsequent research is required to determine if tidal volume is specifically responsive to shorter light durations and the extent to which a 3h light stimulus may be “masking” the tidal volume response. This work expands upon our current understanding of respiratory physiology to include light duration as a key variable affecting daily breathing.

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Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

Location: SDCC Halls B-H

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Program #/Poster #: PSTR081.10/U6

Topic: G.06. Autonomic Regulation

Support: MF-2204-02555
NIDDK-P30-DK040561

Title: Ep3r-expressing mnpo neurons are required for the generation of hot flushes

Authors: *N. D. LYNCH[1], L. ANGENENDT DA COSTA[2], A. KELLY[1], C. CUILTY VILLARREAL[1], C. E. MAHONEY[1], C. B. SAPER[1], N. L. MACHADO[1];
[1]Neurol., Beth Israel Deaconess Med. Ctr. and Harvard Med. Sch., Boston, MA; [2]Univ. of São Paulo, Ribeirao Preto, Brazil

Abstract: The decrease of circulating estrogens in perimenopausal women is accompanied by symptoms (VMS), also called hot flushes. These events are marked by a sudden activation of heat dissipation mechanisms (flushing) accompanied by a sensation of warmth. Previous studies have indicated that estrogen decline causes ARC^{Kndy} cells to release increased levels of neurokinin-B (NKB), a neuropeptide that acts on neurokinin 3 receptors (NK3R). NK3R is widely expressed in the brain, but its expression in the median preoptic nucleus (MnPO) is thought to underlie the generation of VMS. However, a key unresolved question is whether NK3R-expressing MnPO neurons ($MnPO^{NK3R}$) are thermoregulatory cells, as required to elicit VMS. Prior work from our group has identified the prostaglandin EP3 receptor-expressing MnPO ($MnPO^{EP3R}$) neurons as a key population for thermoregulatory control. We have now used in situ hybridization to identify a subset of $MnPO^{EP3R}$ neurons (~ 31%) that express NK3R, suggesting that the $MnPO^{EP3R/NK3R}$ neurons are a crucial link between thermoregulatory control and estrogen-mediated effects on body temperature regulation that underlie hot flushes. To test this hypothesis, we first used a calcium indicator with *in vivo* fiber photometry as a proxy for changes in neuronal activity. We found that $MnPO^{EP3R}$ neurons are strongly recruited after subcutaneous injection of Senktide (Sk), an NK3 receptor agonist that causes VMS-like responses (DF/F -0.006 ± 0.005 SEM saline vs DF/F 0.041 ± 0.008 SEM Sk, n=3 and 4, respectively). Then, we tested the necessity of $MnPO^{EP3R}$ neurons for VMS generation. Compared to intact wild-type controls, mice with cell-specific ablation of $MnPO^{EP3R}$ neurons had significantly reduced effects of both Sk-induced heat dissipation and body temperature reduction (-3.2°C ± 0.1°C SEM WT Sk Tb vs. -0.2°C ± 0.2°C SEM $MnPO^{EP3R}$-ablated mice Sk Tb, n=4 in each group). Our findings suggest that NK3R and EP3R co-expression marks a distinct MnPO neuronal population of thermoregulatory cells essential for VMS. Identifying these neurons and their role provides crucial insight into central mechanisms responsible for the development of hot flushes and offers a potential therapeutic target for VMS treatment.

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Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

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Program #/Poster #: PSTR081.11/U7

Topic: G.06. Autonomic Regulation

Support: GZNL2024A02001
GZNL2025C02012

Title: A midbrain circuit for cough suppression

Authors: W. ZHANG[1], T. WANG[2], X. XU[3], X. NIE[3], J. YANG[4], ***F. LUO**[3];
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Abstract: The cough reflex is a vital innate defense mechanism of the airway, subject to both facilitation and suppression. While recent advances have illuminated central neural circuits that promote coughing, the mechanisms underlying cough suppression remain poorly understood. Here, we utilized whole-body plethysmography, audio, and video tracking to monitor cough-like behaviors in conscious mice. We identified the midbrain periaqueductal gray (PAG) and cuneiform nucleus (CnF) as key regulators of cough suppression. Using a combination of chemogenetic and optogenetic approaches in mice, we demonstrate a bidirectional modulation of cough sensitivity within these regions: silencing neuronal activity increases cough frequency, whereas increasing their activity suppresses coughing. Anatomical tracing reveals that both PAG and CnF project to different downstream targets implicated in respiratory control. To further delineate the underlying mechanisms, we are employing circuit-specific tracing and manipulation strategies to resolve the cellular and network-level architecture governing cough suppression. These findings should uncover a previously unrecognized regulatory system for the homeostatic control of cough sensitivity, with potential implications for developing novel antitussive therapies.

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Poster

PSTR081: Thermoregulation, Respiratory Regulation, and Other

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Program #/Poster #: PSTR081.12/U8

Topic: B.06. Intrinsic Membrane Properties and Signal integration

Support: NIH Grant 2R01NS115209-06

Title: Membrane potential responses to cold temperature in Drosophila larval nociceptor neurons

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Abstract: In *Drosophila* larvae, noxious cold temperatures are detected mainly by Class III (CIII) primary sensory neurons. Previously, we demonstrated using extracellular recordings that, in response to rapid cooling, CIII neurons generate an initial high-frequency peak of spiking activity, which often contains bursts, followed by a lower steady-state phase with tonic spiking activity[1]. Here, using intracellular microelectrodes, we investigated the membrane potential activity of CIII neurons and their responses to cold temperatures. At room temperature (20-24 °C), CIII neurons had resting membrane potentials between -55 and -31 mV, with 1-5 mV fluctuations. The neurons sporadically generated spikes, which occasionally formed brief trains of two to five spikes at a frequency of 4-7 Hz. These sporadic bursts were observed in 90% of the neurons. Depolarizing current injections evoked either high-frequency tonic spiking or periodic square-wave bursts, in which high-frequency spikes (>50 Hz) were superimposed on a depolarizing driver potential. In response to a rapid temperature drop from room temperature to 10 °C, CIII neurons depolarized and generated spiking responses, with the highest peak frequency occurring during the cooling phase. The input resistance of CIII neurons was 1-3 GΩ and increased by 50-75% during exposure to cold temperature. In approximately half of the neurons, the square-wave bursts constituted the majority of the spiking activity during the peak response phase. To investigate the mechanisms underlying spiking responses, we updated our previous model[1] by adding a two-state TRP-channel representation with temperature- and voltage-dependent activation, as well as Ca^{2+}-dependent activation and desensitization. The model reproduces the experimentally observed responses to both injected current and temperature drops. It captures the resting membrane potential and low-frequency spiking activity of CIII neurons at room temperature, as well as the waveform features of square-wave bursting under varying current and temperature conditions. The results demonstrate that CIII neurons exhibit three distinct spiking patterns: tonic spiking, sporadic bursts characterized by low-frequency spikes, and square-wave bursts composed of high-frequency spikes. These findings advance our understanding of the membrane mechanisms underlying temperature coding in peripheral sensory neurons. [1] Maksymchuk N, et al. (2022). *Front Cell Neurosci*, 16: 831803.

Disclosures: A. Sakurai: None. **S.M. Korogod:** None. **B. Gifford:** None. **N. Maksymchuk:** None. **D.N. Cox:** None. **G.S. Cymbalyuk:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.01/U9

Topic: G.07. Biological Rhythms and Sleep

Title: Ovariectomy influence on modulation nicotine-seeking behavior in female mice

Authors: *C. J. JUÁREZ-PORTILLA[1], Z. M. ROSAS[2], G. AGUIRRE[1], T. MOLINA-JIMENEZ[1], R. C. ZEPEDA[1], A. MARTÍNEZ[1], R. C. SALGADO[3];
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Abstract: New forms of nicotine intake, such as vaping, have significantly increased over the last decade and, along with traditional smoking, represent a global public health concern. Epidemiological studies indicate that men generally consume psychostimulants at higher rates, while women tend to consume larger doses over shorter periods. Hormonal fluctuations have been shown to play a key role in modulating responses of women to drugs, with estradiol influencing the effects of substances such as methamphetamine, cocaine, and nicotine. In animal models, fluctuations during the estrous cycle are associated with an increased preference for psychostimulants intake in females. Accordingly, females demonstrate greater vulnerability during various stages of the addictive cycle, including acquisition, escalation, extinction, and relapse. Furthermore, the timing of drug access to circadian rhythms appears to influence anticipatory behavior. Preclinical evidence suggests that nicotine disrupts circadian architecture. Based on these observations, our study examined the combined influence of steroid hormones and time-restricted nicotine intake on drug-seeking behavior in intact and ovariectomized female C57BL/6 mice. Animals were nebulized once daily at Zeitgeber Time 4 (ZT4) for 20 minutes over 14 days with either distilled water (control) or nicotine (experimental). Locomotor activity was recorded using wheel revolution sensors. Our results revealed differences in anticipatory activity amplitude and elongation of the metestrus phase, suggesting that nicotine intake plays a significant role in promoting drug-seeking behavior, likely modulated by the estrous cycle.

Disclosures: C.J. Juárez-Portilla: None. **Z.M. Rosas:** None. **G. Aguirre:** None. **T. Molina-jimenez:** None. **R.C. Zepeda:** None. **A. Martínez:** None. **R.C. Salgado:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.02/U10

Topic: G.07. Biological Rhythms and Sleep

Support: Whitehall Foundation
NIH R35GM151020

Title: Species-specific differences in circadian clock function in diurnal and nocturnal mice

Authors: *V. MUHL[1], J. R. JONES[2];
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Abstract: Circadian rhythms in behavior and physiology are coordinated by the brain's central pacemaker, the suprachiasmatic nucleus (SCN). The SCN is a network of neurons that contain "molecular clocks" and exhibit daily rhythms in action potential frequency. We have previously

shown that in the SCN of nocturnal mice, firing rate rhythms can feedback onto the molecular clock and influence its timing. Surprisingly, the day-night difference in SCN firing rate in diurnal species is similar to that of nocturnal species. However, the interaction between firing rate rhythms and the molecular clock in the SCN of diurnal species is unknown. We hypothesized that species-specific differences in this interaction in the central pacemaker may contribute to temporal niche preference. To test this, we first transfected ex vivo SCN slices from diurnal African striped mice with an adeno-associated virus (AAV) encoding Per2-ELuc, a bioluminescent molecular clock reporter. We continuously measured bioluminescence over multiple days to identify, for the first time, molecular clock rhythms in the SCN of a diurnal animal. We found that the timing of these molecular clock rhythms did not differ between laboratory and striped mice. Next, we transfected striped mouse SCN slices with AAVs encoding Per2-ELuc and the optogenetic construct ChrimsonR. We then measured molecular clock reporter bioluminescence while using optogenetics to acutely increase SCN neuronal activity at different times of day. Our preliminary analysis suggests that the phase response curve of the SCN molecular clock to timed optogenetic manipulation differs between laboratory and striped mice. Finally, we transfected striped mouse SCN slices with AAVs encoding the genetically encoded calcium indicator GCaMP6s. Using time-lapse confocal imaging, we measured neuronal activity from individual SCN neurons every 15 min for 6 days. We identified daily waves of synchronized neuronal activity that spread from the dorsomedial to ventrolateral SCN in striped mice. These waves spread in a pattern similar to, but distinct from, that previously reported in laboratory mice. Together, these findings demonstrate that while SCN circuits are similar between diurnal and nocturnal animals, they have important differences, offering new insights into circadian rhythm regulation and its implications for disease in diurnal species like humans.

Disclosures: V. Muhl: None. **J.R. Jones:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.03/U11

Topic: G.07. Biological Rhythms and Sleep

Title: Neurophysiological Signatures of Hibernation in the Forebrain of Syrian Hamsters

Authors: *R. Y. SATO[1], Y. MUKAI[1], S. DAGHER[2], S. ISHIYAMA[3], Y. YAMAGUCHI[4], H. NORIMOTO[1];
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Abstract: Hibernation is a survival strategy employed by certain animals to withstand harsh environmental conditions, such as extreme drops in ambient temperature or food shortages. During hibernation, animals exhibit a systemic suppression of metabolism and energy expenditure, by a torpid state with a profound decrease in body temperature. In small-bodied hibernators, such as ground squirrels and Syrian hamsters, the hibernation period comprises cycles of two distinct phases: deep torpor (DT) and periodic arousal (PA). During the DT phase, animals remain in a torpid state for several days to weeks with significantly reduced body temperature and metabolic activity. In contrast, the PA phase involves the restoration of body temperature to euthermic levels, accompanied by behaviors such as sleep. The PA phase typically lasts for approximately one day. While previous research has explored the mechanisms and functions underlying both DT and PA phases, many key questions remain unanswered, including: 1. What are the neural activities characteristic of hibernation? While the cortical surface may appear silent during DT, could neuronal activity be detected in deeper cortical layers or the hippocampus? 2. What is the purpose of PA? One hypothesis regarding the purpose and significance of PA is that animals "wake up to sleep", but it requires further evidence to be substantiated. 3. Does the forebrain possess a system capable of retaining memory during hibernation? To address such questions, we need a better understanding of the neural correlates of hibernation. We took advantage of the unique ability of Syrian hamsters to hibernate at any time of the year. Using multichannel silicon probes, we succeeded in recording neuronal activity from the hippocampus and the deep layer of the cortex in hibernating animals. In this poster session, we aim to present the latest electrophysiological data and share our experiences, including numerous challenges and setbacks encountered during the experiments.

Disclosures: R.Y. Sato: None. **Y. Mukai:** None. **S. Dagher:** None. **S. Ishiyama:** None. **Y. Yamaguchi:** None. **H. Norimoto:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.04/U12

Topic: G.07. Biological Rhythms and Sleep

Support: JSPS,(24K02065 to KK)

Title: Investigation of the effect of illuminance on the resynchronization of sleep-wake rhythm

Authors: *Y. SUGIMOTO[1], K. KOZUKA[2], R. EGAMI[2], J. TOMITA[2], K. KUME[2];
[1]Nagoya city university, Grad. Sch. of Pharmaceut. Sci., Nagoya-shi,Aichi-ken, Japan; [2]Nagoya City Univ., Nagoya, Japan

Abstract: The two-oscillator model has been proposed as a hypothesis that differs from the two-process model that has been frequently used to explain sleep-wake rhythms. In the two-oscillator model, the sleep-wake rhythm is explained by the interaction between the suprachiasmatic

nucleus (SCN)-dependent rhythm (oscillator I), which is regulated by light and controls circadian rhythms, and the SCN-independent rhythm (oscillator II), which is regulated by the dopamine system, etc. The two rhythms are normally synchronized, but under special circumstances, they become desynchronized, which is called internal desynchronization.In the mouse model of jet lag by phase advance, the circadian and sleep-wake rhythms gradually become synchronized over several days. However, in our laboratory experiments, we observed many individuals whose sleep-wake rhythms immediately synchronized after phase advance, in contrast to previous reports. These individuals may have undergone internal desynchronization, in which the sleep-wake rhythm dissociates from the SCN rhythm. To investigate the cause of this phenomenon, we examined the differences between individuals that gradually synchronized and those that immediately synchronized. We found that the main cause was the large difference in the intensity of illuminance between light and dark conditions.As a result, we succeeded in creating two types of jet-lag model mice with different entrainment speeds by adjusting the intensity of illuminance. The rapid change in the intensity of illuminance during the switch from light to dark causes the mice to wake up and experience a masking phenomenon, but the mechanism by which the SCN-independent sleep-wake rhythm rapidly synchronizes to the new light/dark environment is unknown. In the future, we will administer drugs to these model mice and examine changes in the rate of entrainment to the new light/dark environment.This study will be a stepping stone to elucidate the effects of the external environment on circadian and sleep-wake rhythms, treatment mechanisms of jet lag, and internal desynchronization.

Disclosures: Y. Sugimoto: None. **K. Kozuka:** None. **R. Egami:** None. **J. Tomita:** None. **K. Kume:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.05/U13

Topic: G.07. Biological Rhythms and Sleep

Support: NIDA R01DA039865
NIDA F32DA060613

Title: Circadian rhythms in cholinergic interneurons and the impact of nicotine exposure

Authors: *T. A. STOWE[1], Y. HUANG[2], C. A. MCCLUNG[4], M. E. JOFFE[3];
[2]Psychiatry, [3]Pharmacol., [1]Univ. of Pittsburgh, Pittsburgh, PA; [4]Psychiatry, Univ. of Pittsburgh Med. Sch., Pittsburgh, PA

Abstract: Nicotine addiction remains a major public health issue, contributing to hundreds of thousands of deaths annually despite decades of research and prevention efforts. As of 2022, ~11% of adults smoked cigarettes, and there is increased use of electronic nicotine delivery systems among both long-term and naïve users, as they are often viewed as “safer” alternatives.

Clinical studies reveal clear circadian patterns in both nicotine consumption and craving, yet chronic nicotine use also disrupts sleep-wake rhythms. Conversely, disruptions in circadian rhythms may heighten vulnerability to nicotine initiation and dependence. Despite this, the mechanisms linking circadian rhythms and nicotine addiction remain poorly understood. Circadian rhythms are present in the mesolimbic dopamine (DA) system, which underlies substance-related behaviors such as drug-taking. For example, diurnal variations in dopamine release and extracellular tone exist in the nucleus accumbens (NAc). The NAc also contains cholinergic interneurons (CINs) that are potent modulators of DA dynamics and motivated behaviors. CIN firing drives acetylcholine (ACh) release onto nicotinic receptors (nAChRs) at DA terminals. However, little is known about the role of circadian rhythms in CINs and the impact of chronic nicotine exposure. Previous research found that there are diurnal rhythms in ACh modulation of DA release. Here, we have expanded from those studies and have determined baseline diurnal rhythms in CIN firing frequency using *ex vivo* electrophysiology in male and female ChAT-gfp mice. Our results found significantly higher CIN firing frequency during the dark cycle compared to the light cycle, specifically in males. Pharmacological methods indicated these rhythms may be mediated through β2-containing nAChRs. Preliminary data also suggest chronic nicotine exposure disrupts these rhythms in CIN activity in males. These findings highlight novel diurnal rhythms and sex differences in CIN activity within the NAc, shedding light on mechanisms potentially central to nicotine addiction and broader substance use disorders (SUDs). Understanding these rhythmic interactions may offer new strategies for personalized treatments targeting circadian disruptions associated with SUDs.

Disclosures: T.A. Stowe: None. **Y. Huang:** None. **C.A. McClung:** None. **M.E. Joffe:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.06/U14

Topic: G.07. Biological Rhythms and Sleep

Support: Northeastern Project-Based Exploration for the Advancement of Knowledge Summit Award

Title: Evaluating the Effect of Timing of Mating on Reproductive Outcomes in Mice

Authors: *C. BLAKE[1], S. ATEN[2], C. B. SAPER[3];
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Abstract: Physiological processes in nearly every species are regulated by the circadian clock. For example, the reproductive system (of females) requires tight temporal organization of estrogen-sensitive neural circuits that allows the hypothalamo-pituitary gonadal (HPG) axis to function properly. Furthermore, growing evidence has shown that the circadian clock is closely

linked to male fertility, given its temporal control over steroid hormone synthesis and secretion. From a behavioral standpoint, unpublished research performed in our (Saper) lab has confirmed that there exists a circadian rhythm in sexual behavior in both male and female mice. Interestingly, this rhythm in sexual behavior overlaps between the sexes, with the 'peak' timing for sexual behavior between **C**ircadian **T**ime (CT) 13-16, and the 'nadir' (low point) between CT4-7. Given that infertility is a global public health issue, especially in individuals who work night shifts, we sought to explore whether the timing in which mating occurs affects the rate (and likelihood) of conception and successful pregnancy. We conducted 1-hr long mating experiments in 4 different cohorts of *WT* male and female mice with aligned sexual behavior rhythms (e.g. male CT16 peak and female CT16 peak) or misaligned sexual behavior rhythms (e.g. male CT16 peak and female CT4 nadir) and monitored outcomes of mating via ultrasound at ~7 and ~13 days post-copulation. All mice received prior sexual experience and were between 14 and 24 weeks of age. Female mice were in estrus at the time of mating, as verified by vaginal swabs and cytological examination or implanted temperature probes. The number of pregnancies carried to term was significantly higher in the cohort in which the male and female were both mated at their sexual peaks, compared to the cohort wherein the male and female were mated at their sexual nadirs. Additionally, we found a difference in pup weight depending on the circadian time of mating. Interestingly, we also found that regardless of the time-of-day in which females were mated, as long as the male was mated during its peak, pregnancies were more likely to carry to term, suggesting that successful conception may be more dependent on male reproductive factors. This insight could be crucial for translational studies that explore whether timed intercourse in humans might help to increase fertility chances.

Disclosures: C. Blake: None. **S. Aten:** None. **C.B. Saper:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.07/U15

Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant 1K01HL145339-01A1

Title: Associations among age, biological sex, perineuronal net plasticity, circadian rhythms, and stress in the Syrian hamster (Mesocricetus auratus)

Authors: *R. A. VILLARREAL[1], L. GARCIA[2], J. LUNA[3], E. I. ALANIZ[2], M. GIL[2,4]; [1]Psychological Sci., Univ. of Texas Rio Grande Valley, Mcallen, TX; [2]Dept. of Psychological Sci., [3]Dept. of Hlth. and Biomed. Sci., [4]Sch. of Med. Inst. of Neurosci., The Univ. of Texas Rio Grande Valley, Brownsville, TX

Abstract: Stress-induced circadian rhythm disruption is linked to metabolic, cardiovascular, and mood disorders. The suprachiasmatic nucleus and other hypothalamic structures play a role in

circadian regulation of behavior, aligning biological processes with environmental light-dark cycles. Acute stress, especially upon waking, can disrupt this cycle and influence mood. Perineuronal nets (PNNs), extracellular matrix proteoglycans surrounding neurons and glia, are involved in plasticity and are present in the hypothalamus, including regions that regulate circadian rhythms and stress, but more research is needed to determine their roles in aging and stress processes. Syrian hamsters (*Mesocricetus auratus)* are an ideal animal model due to their well characterized rhythms and behaviors. Subjects were housed under a 14:10 h light/dark cycle (lights off at 11:00). In experiment 1, qualitative analysis of activity patterns revealed older hamsters exhibited lower activity levels leading to the waking period compared to younger adults. Both age groups showed an increase in activity at wake time. A repeated-measures ANOVA conducted across 24 1-hr time periods identified a significant increase in locomotor activity at the 11:00, 12:00, and 13:00 time periods ($p < 0.05$) as expected. For experiment 2, using a within-subjects design, each hamster underwent both control and stress conditions (30-min restraint before waking), followed by placement in a ANY-maze photobeam array apparatus at 11:00 to track activity (counts, bouts, and total active time) for 2 hrs. Grooming, freezing, and locomotion were scored during restraint, with inter-rater reliability assessed using Cohen's Kappa. Although limited by a small sample size ($n = 3$), observations suggested a trend toward increased activity in the final 15 mins of the post-stress 2-hr activity period. Evaluation of qualitative data indicated more continuous movement in the male following stress exposure, with reduced movement in the female. For experiment 3, a repeated-measures ANOVA focusing on PNN numbers across several brain regions (e.g., PrL, M1, MPO, MnPO, SC, VTA) revealed a significant interaction between age and brain area in a no-stress condition, $F(10, 20) = 3.17$, $p < 0.05$. The Median Preoptic Nucleus showed the greatest age-related difference in PNN numbers. Ongoing PNN analysis will examine whether age influences stress-induced changes in hypothalamic plasticity. We also plan to determine whether other factors, including social experience, influence the relationship among PNNs, stress, and circadian rhythms. These findings may inform neuroplasticity models of stress vulnerability and rhythm disruption.

Disclosures: R.A. Villarreal: None. **L. Garcia:** None. **J. Luna:** None. **E.I. Alaniz:** None. **M. Gil:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.08/U16

Topic: G.07. Biological Rhythms and Sleep

Title: Investigating Hippocampal Contributions to Sleep Disruption in Down Syndrome Mouse Models

Authors: *J. JACKSON[1], J. K. KUSHNER[2], S. ZOU[2], C. BORSKI[2], B. FREIGASSNER[2], P. CAIN[3], C. HOEFFER, Jr.[1];

[1]Integrative Physiol., [2]Inst. for Behavioral Genet., Univ. of Colorado - Boulder, Boulder, CO; [3]Inst. for Behavioral Genet., Univ. of Colorado - Boulder, Boulder, NY

Abstract: Sleep disturbances are a prominent feature of Down syndrome (DS) and are thought to contribute significantly to the cognitive and neurological deficits associated with the condition. While the cortical mechanisms underlying sleep dysfunction have been increasingly explored, the role of hippocampal circuits in these impairments remains less well understood. In this study, we aim to investigate the hippocampal correlates of sleep disruption in the Dp(16)1Yey and Ts66Yah mouse models of DS, employing a multimodal approach that combines chronic EEG/EMG monitoring with in vitro whole-cell patch-clamp recordings from CA1 pyramidal neurons.We hypothesize that DS mice will exhibit altered sleep architecture, including reduced non-rapid eye movement (NREM) sleep and increased sleep fragmentation, particularly during the dark phase, when delta power typically peaks. Additionally, we expect to observe disruptions in the spectral composition of REM sleep, indicative of impaired hippocampal network coordination. At the cellular level, we will assess whether CA1 pyramidal neurons in DS mice display intrinsic excitability changes, such as increased input resistance, depolarized resting membrane potential, and reduced spike frequency adaptation, that potentially reflect sensitization or desensitization of action potential dynamics due to chronic sleep disruption. This study aims to clarify how hippocampal dysfunction contributes to sleep and cognitive phenotypes in Down syndrome (DS) and may inform future therapeutic strategies targeting neuronal excitability and sleep homeostasis.

Disclosures: J. Jackson: None. **J.K. Kushner:** None. **S. zou:** None. **C. Borski:** None. **B. Freigassner:** None. **P. Cain:** None. **C. Hoeffer:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.09/V1

Topic: G.07. Biological Rhythms and Sleep

Support: NIH/NCI Grant # P30 CA062203

Title: Integrated metabolic and circadian profiling in rodent models using breath analysis and telemetry

Authors: *D. OETTLER[1], R. BROCKWAY[2], S. CHUN[3], W. SONG[3], A. VERLANDE[3], S. MASRI[4];
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Abstract: Metabolic disruptions are key features of chronic diseases such as cancer and diet-induced obesity (DIO). We used advanced breath analysis to noninvasively monitor systemic

metabolism in mouse models of lung cancer-induced cachexia and DIO and implantable telemetry to assess physiological rhythms in rats. Using a U-^{13}C glucose tracer with stable isotope sensors, we found increased $^{13}CO_2$ exhalation in tumor-bearing mice—indicating elevated glucose oxidation—while DIO mice exhibited reduced glucose metabolism, consistent with insulin resistance. Telemetry implants enabled 24-hour profiling of cardiovascular parameters, core temperature, activity, and sleep stages. Data revealed robust circadian patterns in blood pressure, heart rate, temperature, and sleep architecture, with expected day-night variability. This dual-platform approach offers a powerful, translational tool to link metabolic shifts with physiological rhythms in disease states and highlights the value of stable isotope use in metabolic measurements and telemetry technologies for integrated phenotyping in the home cage.

Disclosures: D. Oettler: A. Employment/Salary (full or part-time): TSE Systems Inc. **R. Brockway:** A. Employment/Salary (full or part-time): TSE Systems Inc.. **S. Chun:** None. **W. Song:** None. **A. Verlande:** None. **S. Masri:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.10/Web Only

Topic: G.07. Biological Rhythms and Sleep

Title: Uncovering Sleep and Circadian Disruption in TBI Using Wearable Technology and Sleep Deprivation

Authors: *A. POLLATOU[1], E. METZGER[2], J. K. WERNER, Jr.[3];
[1]Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]USUHS, Bethesda, MD; [3]Neurosci, Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Background: Traumatic brain injury (TBI) disrupts sleep and circadian rhythms, worsening cognitive and physiological impairments. Sleep deprivation, common in military and civilian populations, exacerbates these disruptions, yet its effects in TBI remain understudied. Circadian dysfunction is difficult to measure and often mistaken for insomnia, leading to ineffective care. Wearables capture real-world circadian patterns, potentially identifying disruptions missed clinically. This study examines sleep-circadian interactions in sleep-deprived TBI and non-TBI participants. Data collection is ongoing and will be completed by presentation. Methods: Twenty-five participants (N=16 with TBI) were monitored at home for two weeks using the Oura ring. Circadian parameters, including acrophase and mesor, were derived from skin temperature via cosinor analysis. Midsleep time was calculated from sleep data. After 36 hours of sleep deprivation, participants underwent in-lab polysomnography (PSG) to assess sleep architecture, including REM and NREM epochs. Linear regression models examined how TBI moderates relationships between circadian and sleep metrics (beta: interaction effect). Additional analyses explore whether acrophase mediates the association between TBI and sleep quality.

Results: Preliminary findings show TBI participants had significantly earlier circadian timing, with acrophase at 1:55 AM vs. 3:02 AM in non-TBI participants ($p<0.05$). TBI status moderated the acrophase-midsleep relationship (beta=-0.5, p=0.033), suggesting disrupted circadian alignment. While PSG parameters showed no group differences, wearable-derived measures detected disruptions not captured in-lab. Early findings indicate weaker associations between circadian markers and restorative NREM sleep in TBI, suggesting impaired homeostasis.
Conclusions: Initial analysis suggests wearables can detect circadian disruptions in TBI that may be missed clinically. Findings indicate earlier circadian timing and impaired sleep-circadian integration in TBI, particularly post-deprivation. Wearables offer a non-invasive way to identify subtle disruptions with clinical potential. Additional data will clarify whether these relationships persist and their impact on sleep and circadian function in TBI. These results underscore the need for targeted interventions addressing circadian and sleep dysfunction in TBI populations.
Disclaimer: The opinions and assertions expressed herein are those of the author(s) and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences or the Department of Defense.

Disclosures: A. Pollatou: None. **E. Metzger:** None. **J.K. Werner:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.11/V2

Topic: G.07. Biological Rhythms and Sleep

Support: NIH NICHD 5U54HD087101

Title: Impact of light spectrum and intensity on circadian entrainment in mice

Authors: *C. T. CHEN[1], J. R. HARVEY[2], J. KAPLAN[2], V. HSIAO[2], C. A. GHIANI[3], C. COLWELL[4];
[1]Integrative Biol. & Physiol., [3]Pathology, [2]UCLA, Los Angeles, CA; [4]Psychiatry and Biobehavioral Sci., Univ. of California - Los Angeles, Los Angeles, CA

Abstract: The daily cycle of light and dark regulates the sleep/wake cycle of humans and other organisms. However, exposure to light at night has been shown to disrupt these daily rhythms. Specialized photopigments in our eyes, maximally sensitive to blue/green wavelengths and relatively insensitive to red wavelengths of light, detect light and transmit the information to our central clock. In prior work, we found red-enriched light minimizes the negative effects of artificial light at night compared to blue-enriched or white light. In this study, we explored whether different spectra and intensities of light exposure during the appropriate time—namely, the daytime—could improve synchronization of the sleep/wake cycle. C57BL/6 wild-type mice were divided into four cohorts based on lighting conditions: blue-enriched (7000K) or pure red illumination at 50 or 10 lx. Daily locomotor activity was monitored using running wheels. The

experimental design included five phases: entrainment to a 12:12 light/dark cycle, phase shifts (6-hour advance and delay), masking effects with a 1-hour light pulse at ZT 14, and exposure to constant darkness following a light-induced phase delay (15-minute pulse at CT 16). Separate cohorts are being exposed to a skeleton photoperiod and constant light. All animals entrained to a 12:12 light-dark cycle under the four lighting conditions and showed no appreciable differences in key circadian parameters. Strikingly, even the red light at 10 lx was sufficient to entrain the circadian system. On the other hand, blue-enriched light facilitated faster re-entrainment after phase shifts and elicited stronger masking responses. These results demonstrate that while lighting spectrum and intensity minimally affect circadian rhythms under stable conditions, they significantly impact responses to acute environmental changes. These studies will lay the groundwork for exploring therapeutic strategies in disease-specific models with circadian disruptions.

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Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.12/V3

Topic: G.07. Biological Rhythms and Sleep

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Title: Sex and ageing differentially influence circadian and infradian rhythms of mice

Authors: *H. D. PIGGINS[1], P. CHANG[2], T. HITREC[3], C. MUIR[3], S. LACEY[3], M. SOFYANA[3], L. CHROBOK[3];
[1]Sch. of Physiology, Pharmacology, and Neuroscience, Univ. of Bristol, Bristol, United Kingdom; [2]Univ. Col. London, London, United Kingdom; [3]PPN, Univ. of Bristol, Bristol, United Kingdom

Abstract: Intrinsic biological rhythms play a critical role in regulating physiological and behavioural processes, but how sex and age influence these rhythms is underexplored. Here, we comprehensively investigated 24h (daily and circadian) and >24h (infradian; 5-day and 10-day periodicity) rhythms of locomotor and ingestive behaviour in single-housed young (3-7 months) and middle-aged (8-16 months) male and female mice. We report distinct sex- and age-related

effects on behavioural rhythms as well as physiological response to single-housing. Young female mice exhibited variable daily but robust 5-day rhythms in wheel-running activity, while middle-aged female mice had precise daily rhythms but lacked 5-day cycles in wheel-running. Both middle-aged male and female mice ran in their wheels for longer per 24h and engaged in a greater proportion of their daily locomotor and ingestive behaviour during the lights-on phase than their younger counterparts. Few male mice expressed significant 5-day rhythms while many exhibited robust locomotor activity rhythms of 10-day periodicity; neither young nor middle-aged female mice showed 10-day rhythms. In response to single-housing, middle-aged mice lost more body weight than young mice, with weight loss being pronounced in middle-aged female mice. Relationships between the different periodicities in rhythms were more complex in female mice, with this complexity increasing with age. Collectively, these findings reveal that sex and age differentially affect physiological response to single housing as well as a range of periodicities in behavioural rhythms and their interactions.

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Poster

PSTR082: Physiology of Biological Clocks and Rhythms

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Topic: G.07. Biological Rhythms and Sleep

Support: Jacob E. Nyenhuis Faculty-Student Collaborative Grant
Christopher Barney Summer Research Fund

Title: The effects of prebiotics on anxiety, the gut-brain-microbiome axis, and locomotor activity following circadian disruption in rats

Authors: *A. J. GALL, M. O'HARA;
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Abstract: Circadian rhythms are endogenous, internally driven cycles of biological, psychological, physiological, and behavioral processes that occur approximately every 24 hours and allow organisms to function optimally in a dynamic world (Foster, 2020). Disruptions to circadian rhythms, such as extended periods of sleep deprivation, shift work, or jet lag, have significant consequences for health (Potter et al., 2016). Prebiotics, agents that stimulate the growth of bacteria in the gut, have emerged as a potential method of improving sleep following sleep disruption (Bowers et al., 2022); however, the role of prebiotics on circadian health is still limited. Our lab recently found that commercially available prebiotics, a blend of galactooligosaccharides (GOS) and beta glucan, are beneficial for healthy, naïve Sprague-Dawley rats by significantly reducing anxiety-like behaviors (Gall & Griffin, 2021). Building on these findings, the current study aims to isolate these effects to GOS by using a commercially

available prebiotic, Bimuno GOS, and to examine how anxiety, the gut-brain-microbiome axis, and locomotor activity are affected by stress-induced changes using a 2-week constant light protocol. These constant lighting conditions are expected to induce circadian disruptions, anxiety, and stress, but to a lesser extent than other more invasive protocols that have been used in previous research. We used the Open Field Test, Elevated Plus Maze, and Light-Dark Box to measure anxiety-like behavior, the Morris Water Maze to measure short-term memory, and infrared (IR) sensors placed on top of the home cage to measure locomotor activity, circadian disruptions, and reentrainment following experimentally induced jet lag conditions. We hypothesized that anxiety-like behavior would be mitigated by use of prebiotics. In addition, we hypothesized that the gut-brain-microbiome axis disruption would be mitigated by the use of prebiotics, which was examined by analyzing corticosterone levels in fecal matter, along with cFos activation in the hypothalamus, suprachiasmatic nucleus (SCN), hippocampus, amygdala, and paraventricular nucleus following a light pulse. Finally, we hypothesized that prebiotics would allow rats to reentrain faster to experimentally induced jet lag conditions. Data collection for this experiment is currently ongoing. We anticipate that these data will shed light on the role of prebiotics on circadian health and may lead to new therapeutic approaches for individuals suffering from sleep or circadian rhythm disruptions.

Disclosures: A.J. Gall: None. **M. O'Hara:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

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Topic: G.07. Biological Rhythms and Sleep

Support: VA Merit Award 3BX003431 to Dr. Michael J. McCarthy

Title: Modeling circadian rhythm disruption in patient derived stem cell models

Authors: *J. BURNS[1], K. E. ROHR[1], M. J. MCCARTHY[2];
[1]Univ. of California San Diego, San Diego, CA; [2]Psychiatry, VA San Diego Healthcare Syst., San Diego, CA

Abstract: Altered circadian rhythms represent a key diagnostic feature of bipolar disorder (BD). Additionally, genetic studies have found overlap between genes associated with chronotype and circadian rhythms and BD risk, further suggesting that circadian rhythm abnormalities are a key feature of BD pathophysiology. Here, we demonstrate how patient derived stem cells can be used to study circadian rhythm alterations in BD and highlight how this model can be used to investigate the contribution of genetic risk to circadian phenotypes. Induced pluripotent stem cells (iPSCs) have been generated from two cohorts: one consisting of 11 individuals from a retrospective lithium response study and another from 11 individuals belonging to an Old Order Amish pedigree with detailed genomic information available. iPSCs will be differentiated into

glutamatergic neurons and transfected with a *Per2-luciferase* reporter, allowing for the longitudinal measurement of circadian rhythms across 5-7 days. We find a loss of synchrony across neurons in BD as well as selective changes in rhythm amplitude that correspond to lithium responsiveness. Moreover, we discuss how this model can be used to study the effect of genetic risk for BD on circadian rhythms parameters using calculated polygenic risk scores and the presence of variants previously linked to BD in this population. This works demonstrates how real-time measurements of circadian rhythms in clinically relevant cell types, coupled with traditional methods, can provide novel insights into BD pathophysiology. Moreover, the investigation into the relationship between genetic variants and circadian rhythm disruption has the potential to uncover novel personalized treatment strategies for BD.

Disclosures: J. Burns: None. **K.E. Rohr:** None. **M.J. McCarthy:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.15/V6

Topic: G.07. Biological Rhythms and Sleep

Title: Tryptophan photoproducts and their effects on behavior and circadian rhythms

Authors: *A. HINDELEH[1], A. ALACHKAR[2];
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Abstract: Tryptophan (Trp) is an essential aromatic amino acid and precursor to neuroactive metabolites such as serotonin and melatonin, which regulate mood, sleep, and circadian rhythms. Upon exposure to ultraviolet light, Trp can also be converted into a variety of photoproducts with known activity at the aryl hydrocarbon receptor (AhR), a key modulator of immune and neural function. While peripheral effects of Trp photoproducts have been linked to circadian regulation, their influence on central circadian mechanisms and behavior remains poorly understood. We hypothesize that Trp photoproducts cross the blood-brain barrier (BBB) and modulate circadian rhythms by acting on central circuits. To test this, we administer specific Trp photoproducts to mice and assess their effects on rhythmic behaviors, including wheel-running activity and feeding patterns, under light-dark and constant darkness conditions. Liquid chromatography-mass spectrometry (LC-MS) is used to quantify brain levels of Trp metabolites and correlate these with circadian behavioral changes. We examine both short-term (1-3 days) and long-term (1-2 weeks) effects to capture transient disruptions and sustained adaptations in circadian function. These findings provide new insight into how light-derived Trp metabolites influence brain circadian rhythms and behavioral outputs, offering a mechanistic link between environmental exposure, metabolism, and daily physiological regulation.

Disclosures: A. Hindeleh: None. **A. Alachkar:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant R01GM143545

Title: Spatiotemporal gating of photic processing in the central circadian clock is regulated by the local network

Authors: *S. A. MCGRAW, K. C. BENTON, M. ZURSIN, R. C. TWINING, I. A. SAVTCHOUK, J. A. EVANS;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Daily rhythms facilitate adaptive homeostasis by aligning internal physiology with local conditions. Critical for this process, photic entrainment of circadian rhythms requires daily resetting of the central clock in the suprachiasmatic nucleus (SCN). Although retinal and intracellular mechanisms inducing SCN resetting are well established, it remains unresolved how SCN neurons process and encode photic cues as a network. Defining SCN visual circuits is an important area of research given the growing body of evidence linking aberrant light exposure to increased risk of physiological and neurological pathology. To better understand the kinetics of SCN photic processing, here we monitor calcium responses to stimulation in real time using ex vivo 2-photon imaging. Aligned with previous work, basal [Ca2+]i was higher in SCN neurons during the day and SCN photic responses were larger at night, confirming both daily rhythms are preserved ex vivo. Additionally, we find that cellular responses to photic stimulation varied among SCN neurons located in different subdomains, with network-level patterning that was stereotyped across individuals and independent of time of day. Using optogenetic and pharmacological approaches, we show that spatiotemporal gating of photic sensitivity is driven by both pre- and post-synaptic mechanisms. Further, we demonstrate that network-level patterning of photic processing is regulated by cellular differences in glutamate receptor composition and sensitivity to network-based signals that repress cellular responsiveness. This work provides new insight into the functional architecture of central circadian circuits that process light. Further work advancing our understanding of this visual response can ultimately inform how bright light during the day provides health benefits whereas mistimed light at night increases disease vulnerability.

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Poster

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant 1RF1DA056394-01

Title: Characterizing How Breathing and Whisking Phase Impact Decision-making

Authors: *Y. LIAO[1], J. GONZALEZ[2], Z. S. CHEN[3];
[1]Ctr. of Data Sci., New York Univ., New York City, NY; [2]Dept. of Psychiatry, New York Univ. Grossman Sch. of Medicine, New York, NY, USA, New York, NY; [3]New York Univ. Grossman Sch. of Med., New York, NY

Abstract: A growing body of evidence suggests that breathing and whisking rhythms play a significant role in brainwide activity, extending well beyond early sensory areas. While these rhythms have been proposed to contribute to cognitive processes such as timing task execution and decision-making, their underlying mechanisms remain poorly understood. In this study, we aim to address this gap by utilizing the open dataset from the International Brain Laboratory. Specifically, we investigate how the phase of these orofacial rhythms influences the performance of a controlled decision-making task in mice. Additionally, we analyze single-unit neuronal recordings to uncover the brain-wide neuronal patterns that support such coordination.

Disclosures: Y. Liao: None. **J. Gonzalez:** None. **Z.S. Chen:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

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Program #/Poster #: PSTR082.18/V9

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R01AG078758
NSF GRFP award

Title: Sex-specific circadian dysregulation of synaptic rhythms in a 3xTg-AD mouse model of Alzheimer's disease

Authors: *B. N. ROUTH[1,2], L. DOAN[2], J. GOEWEY RUIZ[2], C. GIBSON[2], K. BELL[2], L. M. INCE[2], H. A. HOFMANN[3], R. A. MANGIERI[2,1], L. K. FONKEN[2,1];
[1]Interdisciplinary Neurosciences Program, [2]Div. of Pharmacol. and Toxicology, [3]Dept. of Integrative Biol., The Univ. of Texas at Austin, Austin, TX

Abstract: Sleep disruptions are among the earliest behavioral manifestations of Alzheimer’s disease (AD), implicating circadian dysfunction in early disease etiology. Despite this

association, it is unclear how circadian dysregulation might promote neurodegeneration and contribute to disease progression. In healthy individuals, synaptic excitation-inhibition (E-I) balance oscillates across the 24-hr day, and circadian rhythms in E-I balance may temporally constrain excitation and protect against neurotoxicity. Here, we hypothesized that hippocampal E-I balance becomes irregular in early AD pathology, and that restoring synaptic rhythms could protect against neurodegeneration and cognitive deficits. To test this, we assessed synaptic rhythms in the hippocampus of 6-9 mo C57BL/6 (control) and 3xTg-AD mice using transcriptomics and *ex vivo* electrophysiology. RNA sequencing from male and female hippocampal tissue collected across 6 times of day showed robust gene expression rhythms in excitatory and inhibitory synaptic pathways, which were phase-shifted in 3xTg-AD females compared to controls. Voltage clamp recordings from CA1 pyramidal neurons demonstrated that 3xTg-AD females exhibited time-of-day dependent shifts in functional E-I balance, primarily due to changes in rhythmic inhibition. To strengthen diurnal rhythms and potentially restore appropriate time-of-day E-I balance, in a separate cohort of mice, we implemented a time-restricted feeding schedule, in which food was offered for only 8 hours during the animal's active phase to serve as an additional circadian timing cue. After 6 weeks of time restricted feeding, female 3xTg-AD mice improved spatial memory in a Barnes maze compared to mice maintained on *ad libitum* feeding. We further plan to perform transcriptomic and immunohistochemical analyses to determine whether time-restricted feeding restored the temporal regulation of excitation and inhibition in the 3xTg-AD hippocampus. Our findings uncover sex-specific dysregulated rhythms at the synaptic level in early AD pathology and highlight the potential of daily eating and fasting rhythms in the maintenance of synaptic balance. Further, our data suggest that the effectiveness of synapse-targeting AD pharmaceuticals could depend on sex and time-of-day, warranting further investigation on circadian strategies for AD treatments.

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Poster

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Topic: G.07. Biological Rhythms and Sleep

Support: Whitehall Foundation
NIH R35GM151020

Title: Circadian Control of Dopamine Release and Reward-Seeking Behavior in the Mesolimbic Pathway

Authors: *J. N. COOK;
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Abstract: The circadian system is an essential regulator of reward-related neural function and behavior. Through proper timing of biological activity, the circadian system promotes survival by calibrating when animals seek out rewards such as food sources, mates, and safety. Dopamine, a critical neurotransmitter for driving reward-related behaviors, is heavily regulated by the transcription of circadian clock genes in dopamine neurocircuitry. Here, dopaminergic neurons in the ventral tegmental area (VTA) release dopamine into the nucleus accumbens (NAc) to drive reward-seeking behavior. We hypothesized that circadian clock gene expression in VTA dopamine neurons drives rhythms in dopamine release and, in turn, reward-seeking behavior. To test our hypothesis, we used CRISPR/Cas9 technology to selectively ablate the molecular clock in VTA dopamine neurons in cre-dependent Cas9 transgenic mice. We then combined automated delivery of an hourly sucrose reward with long-term fiber photometry to record dopamine release in tandem with reward-seeking behavior over several days in normal 12:12 light:dark cycle followed by several days in constant darkness. We found that circadian clock gene ablation increased reward-seeking behavior and disrupted circadian rhythms in both dopamine release and behavior, highlighting the critical role of circadian timing in reward circuits. Through advancing our fundamental understanding of how the mammalian brain orchestrates proper function of reward-driven behavior, this study provides a framework for investigating maladaptive reward behaviors, a clinically relevant phenotype in neuropsychiatric diseases like addiction and bipolar disorder.

Disclosures: J.N. Cook: None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant R35GM151020; Whitehall Foundation

Title: The hierarchical control of the dorsomedial hypothalamus circadian circuit

Authors: *N. KEENE, F. MOHAMMADPOURMIR, J. JONES;
Biol., Texas A&M Univ., College Station, TX

Abstract: The dorsomedial hypothalamus (DMH) encodes circadian information from the central biological pacemaker, the suprachiasmatic nucleus (SCN), to regulate rhythmic behaviors including locomotion, sleep, and feeding. Given that DMH lesions disrupt locomotor rhythms, we hypothesized that the DMH and its connection to the SCN are crucial for maintaining rhythmic behavior. To test this, we used CRISPR/Cas9-mediated genome editing to selectively ablate the core clock gene Cry1/2 in DMH neurons in male and female mice. We housed mice under a 12:12 light/dark cycle followed by constant darkness (DD) to analyze free-running locomotor activity. We observed that mice with DMH-specific Cry1/2 ablation retained rhythmic

activity, suggesting that intact DMH neurons can encode SCN input even without their own molecular clock function. Following this, we investigated the SCN>DMH communication pathway using retrograde viral tracing. We injected an AAV encoding retrograde virus unilaterally into the DMH and identified a population of neurons within the SCN that projects directly to the DMH. Furthermore, lesioning and optogenetically entraining this SCN subpopulation in DD show altered offsets of locomotor activity, indicating a critical role of the SCN-DMH pathway in timing activity cycles. Together, these findings highlight the DMH's hierarchical position in circadian regulation and suggest that this neural pathway shapes rhythmic behaviors.

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Poster

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH R35GM151020
Whitehall Foundation

Title: The SCN regulation of downstream neural rhythms via a basal forebrain relay

Authors: *F. MOHAMMADPOURMIR;
Texas A&M Univ., College Station, TX

Abstract: Circadian rhythms in behavior are synchronized by the brain's central circadian pacemaker, the suprachiasmatic nucleus (SCN). These behaviors are generated by brain circuits that have their own internal circadian clock. More complex behaviors, such as learning and memory, also show daily rhythms that depend on the SCN and local clocks. The SCN lacks direct anatomical connections to learning and memory-associated regions like the hippocampus, but may exert influence through its connectivity with the basal forebrain (BF). Cholinergic neurons in the BF release acetylcholine onto these regions to regulate learning and memory and thus are a promising link between the SCN and cognitive behavioral outputs. SCN-BF connectivity was shown in hamsters, However, whether this SCN-BF pathway is evolutionarily conserved in mice is unknown.To address this, we performed a directional circuit mapping using both anterograde and retrograde viral tracing approaches in wild-type mice and we identified direct projections originating from a distinct population of SCN neurons. Surprisingly, we also observed direct cholinergic projections from the BF to the SCN. We therefore hypothesized that the SCN and cholinergic neurons in the BF communicate bidirectionally to enhance the robustness of acetylcholine release rhythms and, consequently, daily rhythms in downstream brain regions. To test this hypothesis, we first aimed to understand if acetylcholine release from the BF could alter SCN neuronal activity. To test this, we injected a red-shifted genetically-

encoded calcium indicator jRCaMP1b bilaterally into the SCN of Chat Cre; Ai32 mice that express the optogenetic construct channelrhodopsin in cholinergic neurons. We prepared acute SCN slices at zeitgeber time ZT6 and ZT18 and recorded RCaMP fluorescence before and after optogenetically stimulating (470 nm, 8 Hz, 7.5 min) cholinergic terminals in the SCN. We found that SCN neurons exhibit distinct responses to acetylcholine release: subsets of neurons were excited by, inhibited by, or did not respond to, cholinergic stimulation. These preliminary findings suggest that acetylcholine release from the BF onto the SCN may alter SCN firing rate rhythms. This could provide a mechanism for ensuring SCN output rhythm robustness to ultimately synchronize and optimize memory processes in distant brain regions.

Disclosures: F. Mohammadpourmir: None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

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Program #/Poster #: PSTR082.22/W2

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R35GM151020
Whitehall Foundation

Title: Circadian regulation of neuronal activity in the locus coeruleus-medial prefrontal cortex pathway

Authors: *S. SWECK;
Texas A&M Univ., College Station, TX

Abstract: In the brain, circadian rhythms are generated by molecular clocks that regulate physiology and behavior across the 24-hour cycle. While these rhythms are well-characterized in simple behaviors such as locomotion and feeding, their influence on complex behavior circuits remains poorly understood. The locus coeruleus (LC) and medial prefrontal cortex (mPFC) form a bidirectional circuit that plays a key role in spontaneous transitions between homecage behaviors, yet it is unknown whether this network undergoes circadian modulation. We hypothesized that neural activity within the LC-mPFC circuit is regulated by communication between local molecular clocks to modulate time-of-day differences in behavioral transitions. To test this, we used long-term fiber photometry to record calcium dynamics in both the mPFC and LC noradrenergic terminals within the mPFC across multiple days in freely behaving mice. Male and female mice were injected with GCaMP7s in either the LC or mPFC and implanted with an optical fiber in the mPFC. Animals were recorded under both a normal 12:12 light:dark cycle and in constant darkness to distinguish circadian rhythms from light-driven rhythms. We found that both mPFC activity and LC-mPFC projection activity exhibit robust ~24-hour rhythms that persist in constant darkness and peak in mid- to late- subjective day. These findings provide the first evidence that the LC-mPFC circuit is subject to circadian regulation in vivo. Ongoing work

aims to determine whether local molecular clocks within the LC are necessary to sustain these rhythms, offering insight into how circadian disruption might impair executive function in neuropsychiatric disease.

Disclosures: S. Sweck: None.

Poster

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Topic: G.07. Biological Rhythms and Sleep

Support: 5R21MH131527

Title: Altered lighting conditions elicit sex-specific behaviors in diurnal grass rats

Authors: *J. HARTNER[1], N. MUSCAT[2], D. ZUTSHI[3], L. YAN[5], B. O. WATSON[4];
[1]Univ. of Michigan Neurosci. Inst., Ann Arbor, MI; [2]Univ. of Michigan - Ann Arbor, Ann Arbor, MI; [4]Dept. of Psychiatry, [3]Univ. of Michigan, Ann Arbor, MI; [5]Psychology, Michigan State Univ., East Lansing, MI

Abstract: Circadian rhythms are crucial to daily biological functions, and disruptions or alterations to these rhythms are implicated in changes to brain health seen in neuropsychiatric disease states. Cognitive functions such as attention, choice, and preference-related behaviors are modulated by circadian rhythms and disrupted in mood disorders such as Seasonal Affective Disorder (SAD) and Major Depressive Disorder (MDD). These neuropsychiatric diseases can be induced or worsened by light timing alterations and can also be treated with circadian-timed bright-light therapy, suggesting modulatory effects of light timing and degree on mood and behavior. However, efforts to understand daily biological rhythms are hindered by the fact that commonly used laboratory rodents are nocturnal, which creates a translational gap: a lack of detailed neurophysiologic knowledge of effects of light on diurnal brains.
The Nile grass rat (Arvicanthis niloticus) is diurnal in the wild and has more recently been shown to exhibit diurnal patterns of sleep, locomotor activity, mating behavior, body temperature and secretion of hormones in the laboratory, establishing it as a good rodent model for circadian studies. Characterizations of grass rat circadian behavior are promising, but sex-specific behavioral responses in various lighting conditions have not been examined. This is especially important given the contribution of circadian rhythms and light to mental health, and the known human sex differences in susceptibility to MDD and SAD.
This project aims to reveal the rhythms of daily activity in diurnal mammals in the home-cage in various lighting conditions. We use a novel technique to quantify the daily activity of both male and female diurnal rodents over extended periods of time in various lighting conditions by tracking movement in video frames. We show sex-differences in both circadian patterns of movement as well as in response to light alterations. This work will establish a foundation for

future network function-oriented understanding of the effects of light on mood-based brain circuits in diurnal mammalian brains.

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Poster

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Topic: G.07. Biological Rhythms and Sleep

Support: NIH Grant MH107662

Title: Longitudinal behavioral profiling in mice using the Digital HomeCage: Profiling mouse activity and choice in a controlled environment

Authors: *E. GOLDIEZ[1], N. OGNJANOVSKI[2], K. KNOPF[3], B. O. WATSON[4];
[1]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Pyschiatry, [4]Dept. of Psychiatry, [3]Univ. of Michigan, Ann Arbor, MI

Abstract: Long-term rodent behavioral profiling, similar to "digital phenotyping" in humans, offers a powerful approach to understanding behavioral dynamics and variability. We have developed the “Digital Homecage” system for mice, enabling us to gather multidimensional mouse behavioral data, to discover novel associations between behaviors and pathologic states. We have used this system to collect recordings of naturalistic behavior with minimal experimenter intervention from 48 animals. We investigated variability within and across animals in this dataset. Multiple data streams, including actigraphy, wheel running, and food and drink preferences (regular vs. fatty food, distilled water vs. sucrose water), were collected and analyzed on per-second, hourly, daily, and weekly bases across all 48 C57BL/6J mice. Leveraging big data analytics approaches including UMAP for dimensionality reduction and k-means clustering, we identified three distinct "personality types" emerging from the data, despite genetic, age, and supplier consistency. These clusters highlight inter-individual variation in baseline homecage dynamics including food and water preferences, and wheel-running patterns, with key differences driven by sustained running during specific circadian periods (ZT16-20) and differential consummatory requests. Our findings reveal nuanced behavioral profiles, with mice exhibiting varied preferences for sucrose water and fatty food over time, yet maintaining stable weight. We used a data-driven analytical approach to uncover clusters of behavioral predispositions, providing deeper insights into the biological bases of personality and temperament. These results have significant implications for understanding both normal and pathological behavioral patterns in rodents, offering a foundation for future research into the interplay of genetics, environment, stressors or interventions and behavior in controlled settings.

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Poster

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Eisenberg Depression Center
Gilmore Sleep Fellowship

Title: Daily rhythms drive dynamism in sleep, oscillations and interneuron firing, while excitatory firing remains stable across 24 h

Authors: *D. S. KIM[1], N. OGNJANOVSKI[2], E. GOLDIEZ[4], S. J. ATON[5], B. O. WATSON[3]; [2]Pyschiatry, [3]Dept. of Psychiatry, [1]Univ. of Michigan, Ann Arbor, MI; [4]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [5]Molecular, Cellular, and Developmental Biol., Univ. of Mchigan, Ann Arbor, MI

Abstract: The adaptation to the daily 24-h light-dark cycle is ubiquitous across animal species and is crucial for maintaining fitness. This free-running cycle occurs innately within multiple bodily systems, such as endogenous circadian rhythms in clock-gene expression and synaptic plasticity. These phenomena are well studied; however, it is unknown if and how the 24-h clock affects electrophysiologic network function in vivo. The hippocampus is a region of interest for long timescale (>8 h) studies because it is critical for cognitive function and exhibits time-of-day effects in learning. We recorded single cell spiking activity and local field potentials (LFPs) in mouse hippocampus across the 24-h (12:12-h light/dark) cycle to quantify how electrophysiological network function is modulated across the 24-h day. We found that while inhibitory population firing rates and LFP oscillations exhibit modulation across the day, average excitatory population firing is static. This excitatory stability, despite inhibitory dynamism, may enable consistent around-the-clock function of neural circuits.

Disclosures: D.S. Kim: None. **N. Ognjanovski:** None. **E. Goldiez:** None. **S.J. Aton:** None. **B.O. Watson:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.26/W6

Topic: G.07. Biological Rhythms and Sleep

Support: NIH R01MH126137

Title: Mouse bio-behavioural phenotyping: Using the "Digital Homecage" for long-timescale, high resolution, multi-factor recording of homecage sleep and wake

Authors: N. OGNJANOVSKI[1], A. GHIMIRE[5], D. KIM[1], E. GOLDIEZ[6], ***A. GREEN**[2,1], K. KNOPF[3], B. O. WATSON[4];
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Abstract: Rodent neuroscience tools hold promise for studying brain-based phenomena and disorders, but many such phenomena develop over long periods or require long-duration monitoring to capture. Such slow-developing conditions include chronic stress, circadian disorders, aging, metabolic disorders, and others. In animals, we cannot get verbal responses so behavior is our major measurable brain output, but unfortunately, many such conditions have behavioral phenotypes that are not fully characterized by the standard short-term behavioral tests. When some of those standard tests are given repeatedly to delineate long-term trajectories of brain status, they often show changing behavioral results. On the other hand, non-invasive measures of spontaneous behavior in the homecage can be performed indefinitely for the lifetime of the animal and reveal long timescale dynamics.In an approach called "digital phenotyping", researchers use digital devices such as smartphones or wrist-based trackers to gather long timescale behavior and physiology data in humans. Since rodent behavioral testing typically occurs for only a few minutes or hours per week, rodent neuroscientists stand to gain huge amounts of new information by recording rodent homecage behavior 24-hours per day. The "Digital Homecage" (DHC) framework introduced here closes this gap, by enabling digital phenotyping in mice. It uses a combination of videographic, operant, and wheel-running data to capture 20+ behaviors from a single-housed mouse at sub-second resolution without interruption. It is also designed to allow for future tethered experiments including electrophysiologic recordings, fiber photometry and optogenetics.
Our results show that sucrose water is strongly preferred over regular water, while fatty and regular food are relatively equally requested. We also show that wheel running as well as feeding and drinking behaviors took approximately 14 days to plateau after introduction to the DHC. This finding of multiple days of acclimation has strong implications for the requirement for behaviorists to observe true steady state conditions before making conclusions about steady state behavior. Our automated DHC system will allow major new observations that define long-term behavioral correlates of chronic conditions with a future potential for incorporating brain correlates as well.

Disclosures: N. Ognjanovski: None. **A. Ghimire:** None. **D. Kim:** None. **E. Goldiez:** None. **A. Green:** None. **K. Knopf:** None. **B.O. Watson:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.27/W7

Topic: G.07. Biological Rhythms and Sleep

Support: NIH RF1-AG061831

Title: Regional specialization of rhythmic gene expression across the major spatial axes of the hippocampus and cortex

Authors: *A. GELBER[1], H. ROMERO[2], P. A. DESPLATS[4], E. A. MUKAMEL[3];
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[3]Cognitive Sci., Univ. of California San Diego, La Jolla, CA; [4]Neurosciences and Pathology, Univ. California San Diego, La Jolla, CA

Abstract: Spatial and functional heterogeneity across hippocampal and cortical subregions underlies brain specialization, yet it remains unclear how diurnal transcription varies within these anatomical gradients. We generated a time-resolved spatial transcriptomic dataset by profiling sagittal brain sections from adult mice at 4 Zeitgeber times using the 10X Visium platform. Unsupervised clustering of the entire dataset reliably delineated major hippocampal subfields and cortical layers but did not segregate dorsal from ventral hippocampus or rostral/central/caudal cortex, consistent with the similar cell type composition across these subregions. We manually annotated ST spots corresponding to dorsal and ventral hippocampus and motor (rostral), somatosensory (central) and visual (caudal) cortex, based on anatomical landmarks. We developed a harmonic regression framework based on negative binomial generalized linear model to test differences in diurnal gene expression rhythmicity across subregions. In the cortex, we identified hundreds of genes that were differentially rhythmic when comparing caudal to rostral or central regions. Core clock genes, including Bmal1/Arntl, Dbp, and Per2, had significantly lower rhythmicity in caudal cortex compared with rostral or central regions. Other genes had greater rhythmicity in caudal cortex, including MAPK regulators such as Adcy8 and Elk1. Notably, activity-dependent genes such as Egr1 and Egr3 had a significant difference in the phase of rhythmicity in caudal vs. rostral/central cortex. These genes peaked in their mRNA expression in caudal cortex at around ZT10, near the end of the light phase. By contrast, in motor and somatosensory regions in the rostral/central cortex, Egr1/3 peaked around ZT22 near the end of the dark phase. We identified thousands of differentially expressed genes in dorsal vs. ventral hippocampal, consistent with previously described patterning. Despite this regional specialization of gene expression, harmonic regression demonstrated remarkably conserved rhythmic gene sets in both compartments. The relative amplitude of diurnal expression rhythmicity was strongly correlated in both regions, and there were no genes with a statistically significant difference in rhythmic amplitude or phase (FDR > 0.1 for all genes). These results demonstrate that hippocampal subregions, despite strong static molecular specialization, share a unified circadian framework. By contrast, cortical rhythmic regulation is spatially organized, with distinct

drivers of rhythmic gene expression and opposite phase of activity-dependent genes in the caudal vs. rostral/central regions.

Disclosures: A. Gelber: None. **H. Romero:** None. **P.A. Desplats:** A. Employment/Salary (full or part-time): Co-Director, UC San Diego Center for Circadian Biololgy. **E.A. Mukamel:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.28/W8

Topic: G.07. Biological Rhythms and Sleep

Support: ZonMw Open Competition (grant no. 09120012010051).

Title: Benchmarking the PVN pre-autonomic neurons to find the circadian balance of the autonomic nervous system

Authors: V. RUMANOVA[1,3], D. POORMOGHADAM[3,1], J. B. BERKHOUT[4,5], C.-X. YI[2], A. MAHFOUZ[5,6], O. C. MEIJER[4], ***A. KALSBEEK**[2,3];
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Abstract: The autonomic nervous system (ANS) together with the endocrine system is responsible for organizing homeostasis. The hypothalamic paraventricular nucleus (PVN) is the core brain structure controlling the ANS and endocrine system. Traditionally the ANS is divided in a sympathetic (SNS) and parasympathetic (PNS) branch. The autonomic balance is an important determinant of metabolic health, as imbalance is associated with various pathologies. The suprachiasmatic nucleus (SCN) organizes the daily sleep/wake cycle as well as the autonomic balance that goes along with it. However, it is unclear how the SCN influences the autonomic balance. Activity of PNS and SNS branches is controlled by PVN pre-autonomic neurons that project to resp. brainstem and spinal cord. More insight into how the SCN - PVN projection controls activity of these pre-autonomic neurons is fundamental for a better understanding of the control of the autonomic balance and improving metabolic health. Unfortunately, no clear (molecular) markers are available to identify the pre-autonomic PVN neurons. So far these neurons can only be visualized by retrograde tracing from the spinal cord or brainstem. Recently, we created a detailed atlas of PVN transcriptomic cell types by integrating various mouse hypothalamic single-cell datasets into a PVN single-cell transcriptome atlas (Berkhout et al., 2024). We found 19 neuronal transcriptomic subpopulations in the PVN and some possible markers for the neuro-endocrine and pre-autonomic subpopulations. Next, we performed retrograde tracing from brainstem and spinal cord and applied RNAscope for GFP and 10 of these newly discovered markers to further characterize the identity and localization of

the SNS and PNS pre-autonomic neurons in the PVN. We found 1.415 cells retrogradely labeled with GFP. The presence of RELN appeared to be the best marker to characterize these GFP-labeled pre-autonomic neurons. From the total of 30.523 cells labeled by the RNAscope probes, about 1/3 were pre-autonomic and 2/3 neuro-endocrine. No clear differences were found between the populations of SNS and PNS pre-autonomic neurons, but together they appeared to consist of two main sub-populations, depending on their co-localization with CALB1 (~3.300 cells) or not (~3.900 cells). The neuro-endocrine PVN neurons were best characterized by the expression of SCGN, with the absence or presence of NTNG1 determining their resp. magnocellular (~2.500 cells) or parvocellular (~3.300 cells) nature. RELN cells not expressing NTNG1 (~4.200 cells) and cells expressing neither RELN nor SCGN (~13.300 cells) were mostly located just adjacent to the PVN in the subPVN or Xiphoid nucleus.

Disclosures: V. Rumanova: None. **D. Poormoghadam:** None. **J.B. Berkhout:** None. **C. Yi:** None. **A. Mahfouz:** None. **O.C. Meijer:** None. **A. Kalsbeek:** None.

Poster

PSTR082: Physiology of Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR082.29/W9

Topic: G.07. Biological Rhythms and Sleep

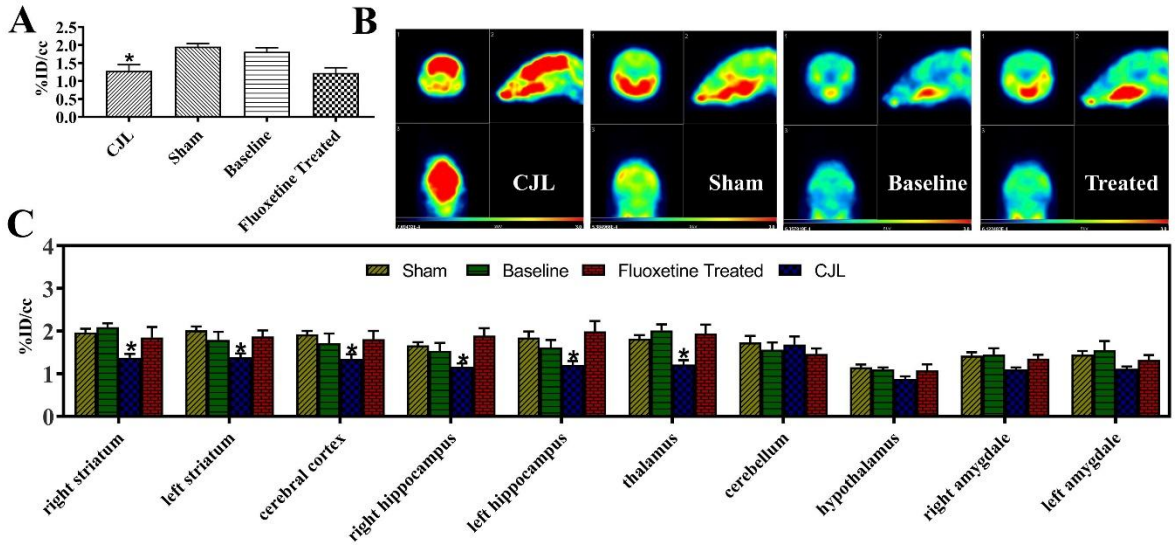
Support: NSFC

Title: Chronic Jetlag Triggers Depression-like Behavior in Mice with Intact Circadian Clock Through Serotonin Pathway Alterations and Clock Gene Dependency

Authors: *S. R. Q. KHAN;
Med. Res. Ctr. of the Second Affiliated Hosp. of Zhengzhou Univ., The Second Affiliated Hosp. of Zhengzhou Univ., Zhengzhou, China

Abstract: Depressive disorders are among the leading causes of disability worldwide and remain difficult to treat effectively. Clinical studies have shown that patients with depression often exhibit disrupted circadian rhythms and sleep disturbances, while circadian misalignment is also considered a contributing factor to the onset of depressive symptoms. In this study, we investigated the mechanisms underlying depression induced by chronic disruption of the light/dark (LD) cycle using a chronic jetlag (CJL) paradigm. Mice were subjected to CJL conditions by advancing or delaying LD cycles by 6 hours every 2-3 days for a period of 90 days. Behavioral assessments, including the tail suspension test (TST), forced swimming test (FST), and sucrose preference test (SPT), revealed a significant increase in depression-like behaviors following CJL exposure. Positron emission tomography (PET) analysis demonstrated a widespread reduction in brain glucose metabolism in CJL-treated mice. RNA sequencing of the nucleus accumbens (NAc) and prefrontal cortex (PFC) identified differentially expressed genes associated with depression, including key regulators of the serotonin signaling pathway.

Supporting this, treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine effectively reversed CJL-induced depressive behaviors. Quantitative PCR further confirmed a significant upregulation of serotonin transporter (Slc6a4) mRNA in the raphe nucleus, a key region for serotonin synthesis. Notably, CJL-induced depression-like phenotypes were attenuated in mice deficient in core circadian clock genes, indicating that an intact molecular clock is essential for the mood-altering effects of circadian disruption.



Disclosures: S.R.Q. Khan: None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.01/W10

Topic: G.08. Food and Water Intake and Energy Balance

Title: Piezo1-mediated ATP release contributes to initiation of swallowing induced by esophageal water stimulation in anesthetized guinea pigs

Authors: *M. YOSHIHARA[1], T. TSUJIMURA[2], B. J. CANNING[3], M. INOUE[1];
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Abstract: Background: Drinking is essential for life, and hydration is linked to osmosensation. While osmosensory mechanisms in the oral cavity and pharyngolarynx have been suggested, the

role of esophageal osmosensation is unclear. Thickened water stimulation of the esophagus induces swallowing in humans, but the effect of hypotonic esophageal stimulation is not fully understood. Hypotonic stimuli can activate Piezo1 channels and induce ATP release in epithelial tissues. In the larynx, ATP receptors are involved in swallowing responses to distilled water (DW). Given these findings, we investigated whether hypotonic stimulation of the esophagus facilitates swallowing and induces ATP release via Piezo1-P2X signaling. Methods: We used 107 male Hartley guinea pigs. In vivo, the electromyogram of the left suprahyoid muscles and esophageal pressure were recorded under urethane anesthesia. Swallowing was induced by intra-esophageal bolus injections (0.05-0.5 ml) of Krebs buffer, DW, ATP (100 μM), or Yoda1 (Piezo1 agonist, 30 μM), and the number of swallows was compared. GsMTx4 (Piezo blocker, 30 μM) and TNP-ATP (P2X blocker, 30 μM) were used to evaluate their effects on DW-induced swallowing. In ex vivo assays, the esophagus was perfused with DW or Krebs (1 cc/min), and luminal ATP was measured. GsMTx4 and ruthenium red (TRP/Piezo blocker, 100 μM) were applied before DW perfusion. Some preparations lacked muscle layers to isolate mucosa. RT-PCR was performed on esophageal mucosa, jugular and nodose ganglia. Extracellular single-unit recordings were made from nodose C-fibers using isolated esophagus-vagus preparations. The lumen was stimulated with 2 ml of DW, αβ-MeATP (P2X agonist, 100 μM), Yoda1, or Krebs. Results: Swallowing was significantly increased by DW, ATP, and Yoda1. GsMTx4 and TNP-ATP significantly suppressed DW-induced swallowing, indicating roles for Piezo1 and P2X receptors. DW perfusion induced higher ATP concentrations in luminal perfusate than Krebs; this was suppressed by RR and GsMTx4. ATP release was also observed in mucosa-only preparations. RT-PCR showed Piezo1 in mucosa and P2X2 and P2X3 receptors in the nodose ganglia. Within the nodose ganglion, both P2X2 and P2X3 receptors were expressed in TRPV1-positive neurons, suggesting their presence in C-fibers. In extracellular recordings, DW, αβ-MeATP, and Yoda1 increased C-fiber action potential firing frequency compared to Krebs. Conclusion: DW stimulation may activate Piezo1 in the esophageal mucosa and induce ATP release, which stimulates P2X receptors in the C-fiber of the nodose ganglion and contributes to the initiation of swallowing.

Disclosures: M. Yoshihara: None. **T. Tsujimura:** None. **B.J. Canning:** None. **M. Inoue:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.02/W11

Topic: G.08. Food and Water Intake and Energy Balance

Support: FAPESP (2015/20500-3
CAPES
CNPq

Title: Fluid-electrolyte balance and anxiety-like behavior in spontaneously hypertensive rats

Authors: E. PEREIRA JUNIOR, A. ZENATTI, L. BARRETTO DE SOUZA, B. GAZITO DIAS, I. MENDES PAZELLO, C. CRESTANI, M. T. MARIN, ***J. MENANI**, L. DE LUCA, C. A. ANDRADE;
UNESP, Araraquara, Brazil

Abstract: Spontaneously hypertensive rats (SHRs), an animal model for the study of primary hypertension, ingest more NaCl and exhibit less anxiety-like behavior than normotensive strains. Here we investigated whether disturbances in the hydromineral balance (such as during dehydration) would change locomotor activity and anxiety-like behavior in SHRs compared to normotensive rats. Behavioral responses of male SHRs (n = 11-12 per group) and normotensive Holtzman rats (n=13-14 per group) were evaluated in the elevated plus-maze (EPM) and in the open field test (OFT) under the following conditions: a) euhydrated; b) after 24 h water deprivation (WD); c) WD followed by 2 h access to water for partial rehydration (PH); and d) after WD-PH followed by 2 h access to both water and 0.3 M NaCl (sodium appetite test). Under euhydrated condition, SHRs had increased time spent in the EPM open arms (49 ± 4%, vs. normotensive: 33 ± 5%) and increased central locomotor activity (23 ± 3, vs. normotensive: 8 ± 2 min). After WD and WD-PR, the behavioral responses in EPM and OFT were not different from euhydrated condition when compared within the same strain. Interestingly, 0.3 M NaCl intake changed behavioral responses compared to euhydrated condition only in SHRs. After 0.3 M NaCl intake, SHRs showed a decreased time spent in the EPM open arms (32 ± 5, vs. euhydrated: 49 ± 4%), a decreased peripheral locomotor activity (79 ± 9, vs. euhydrated: 91 ± 6 min), decreased central locomotor activity (14 ± 2, vs. euhydrated: 23 ± 3 min), and time spent in the center in the OFT (25 ± 2, vs. euhydrated: 46 ± 7 min). The results suggest that WD or WD-PR produced no change in the decreased anxiety-like behavior pattern and increased locomotor activity in SHRs, whereas 0.3 M NaCl intake after WD-PR may attenuate the increased locomotor activity and increase anxiety-like behavior in SHRs.

Disclosures: E. Pereira Junior: None. **A. Zenatti:** None. **L. Barretto de Souza:** None. **B. Gazito Dias:** None. **I. Mendes Pazello:** None. **C. Crestani:** None. **M.T. Marin:** None. **J. Menani:** None. **L. De Luca:** None. **C.A. Andrade:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.03/W12

Topic: G.08. Food and Water Intake and Energy Balance

Support: DK133818 (DD)
IOS-1754878 (MJP)

Title: The impact of drinking on neural activity and glucagon-like peptide-1 receptor immunoreactivity in vasopressin-deficient Brattleboro rats

Authors: *S. A. DAVID[1], J. SIRKIN[2], D. J. BRAKEY[2], M. J. PAUL[1], D. DANIELS[3]; [1]Psychology, [2]Biol. Sci., [3]Dept. of Biol. Sci. and Ctr. for Ingestive Behavior Res., Univ. at Buffalo, Buffalo, NY

Abstract: The vasopressin-deficient Brattleboro rat provides a unique model to study the thirst-specific elements of the glucagon-like peptide-1 (GLP-1) system. Brattleboro rats are polydipsic, and hyper-responsive to the fluid intake suppression caused by GLP-1 receptor (GLP-1R) agonist treatment. To begin to assess neural underpinnings of this increased GLP-1R sensitivity, we examined Fos- and GLP-1R-immunoreactivity after dehydration in Brattleboro and wildtype rats with or without subsequent access to water. Several brain areas that are important for fluid intake control were evaluated, including the subfornical organ (SFO), supraoptic nucleus (SON), nucleus of the solitary tract (NTS), and the anteroventral third ventricle (AV3V). In the SFO, Fos counts differed significantly by genotype only, with Brattleboro rats showing greater activation than wildtypes regardless of hydration state. In the SON, both genotype and condition influenced Fos expression, with higher activation during dehydration in both genotypes, and overall greater activity in Brattleboro rats; however, no interaction was observed. In the NTS, GLP-1R expression showed main effects of both genotype and condition, with increased expression following rehydration and elevated levels overall in Brattleboro rats. NTS Fos counts revealed a significant interaction by which rehydration increased Fos in both genotypes, but did so with a greater magnitude in Brattleboro rats. In the AV3V, GLP-1R expression showed a significant genotype x condition interaction, with Brattleboro rats showing a significant decrease in receptor expression after rehydration. AV3V Fos expression also showed a significant genotype and condition interaction; wildtype rats had reduced activation during dehydration, while Brattleboro rats showed lower activation following rehydration. These results demonstrate brain region-specific modulation of GLP-1R expression and Fos expression by both genotype and hydration status. These findings will enhance our understanding of GLP-1-mediated fluid intake control and its potential dissociation from food intake mechanisms using the Brattleboro rat model.

Disclosures: S.A. David: None. **J. Sirkin:** None. **D.J. Brakey:** None. **M.J. Paul:** None. **D. Daniels:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.04/W13

Topic: G.08. Food and Water Intake and Energy Balance

Support: NIH Grant GR426736

Title: The role of GPR75 in the hypothalamus

Authors: *M. K. BECHER[1], I. MOCCHETTI[2];
[1]Georgetown Univ. Med. Ctr., Washington, DC, DC; [2]Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: This study aims to investigate the role of G-protein coupled receptor 75 (GPR75) in the central regulation of metabolism, with a particular focus on its involvement in proopiomelanocortin (POMC) neurons within the arcuate nucleus of the hypothalamus. Obesity, which is associated with significant global health risks, including cardiovascular disease, stroke, and diabetes, is a result of the increasing imbalance between caloric intake and energy expenditure. Research has identified GPR75 as a potential therapeutic target for obesity treatment. Loss-of-function mutations in the GPR75 gene protect against obesity, as demonstrated in both human genome-wide association studies and GPR75 knockout (KO) mice, which are protected from diet-induced obesity. These mice exhibit lower body weight, increased thermogenesis, and enhanced energy expenditure, suggesting that GPR75 plays a key role in metabolism regulation. The hypothalamus, particularly the arcuate nucleus, regulates energy homeostasis by integrating peripheral signals and controlling feeding behavior and fat breakdown. Our lab's findings show that GPR75 is highly expressed in the hypothalamus, particularly within POMC neurons. POMC neurons, critical for energy balance, promote satiety and thermogenesis upon activation by leptin. These results indicate that GPR75 deletion alters POMC derived neuropeptide expression and leads to changes in neuron activation due to feeding, as seen by c-fos expression, within the arcuate nucleus of the hypothalamus. This study tests the hypothesis that GPR75 deletion alters POMC neuron activity, contributing to the lean phenotype observed in GPR75 KO mice. By exploring the central role of GPR75 in metabolism, this research aims to provide new insights into potential therapeutic strategies for obesity as well as elucidating a novel role of GPR75 in the brain.

Disclosures: M.K. Becher: None. **I. Mocchetti:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.05/W14

Topic: G.08. Food and Water Intake and Energy Balance

Support: Russell Berrie Foundation Fellowship
ADA fellowship 1-25-PDF-64

Title: Regulation of meal size and timing by a brainstem peptidergic population

Authors: *S. CHOWDHURY[1], N. KAMATKAR[1], W. WANG[3], X. WANG[4], A. R. NECTOW[2];
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Abstract: Satiation is a negative feedback process that leads to the termination of a meal. It is dissociable from hunger and satiety signaling and is controlled by a distinct set of neural circuits. While hypothalamic peptidergic neurons play key roles in regulating hunger-satiety dynamics, classic studies utilizing decerebrate rats localized the satiation center in the brainstem. However, the specific cell type, circuit, and mechanistic details concerning how the brainstem regulates satiation are not fully understood. Here, I will describe my recent work, in which we discovered an obligate peptidergic population in the brainstem's dorsal raphe nucleus that regulates satiation. These neurons, marked by the anorectic hormone cholecystokinin (CCK) expression, integrate diverse ingestive cues to regulate meal size and timing. These signals include the sensory properties of food, the bite-by-bite assessment of ingestion, the chemo- and mechano-sensation of food in the stomach, and gut-derived humoral factors. The meal regulatory effect of CCK neurons features a built-in delay and is sustained for tens of minutes even after CCK activity has ceased. Circuit mapping revealed that CCK neurons are ideally positioned to coordinate with other brain areas involved in feeding. This multi-level integration capability, along with its delayed yet sustained functional mode, makes the CCK population an ideal candidate for the long-sought-after "satiation center" in the brainstem.

Disclosures: S. Chowdhury: None. **N. Kamatkar:** None. **W. Wang:** None. **X. Wang:** None. **A.R. Nectow:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.06/W15

Topic: G.08. Food and Water Intake and Energy Balance

Title: Eat this! Caloric restriction without nutrient deficiency influences the neuroinflammatory response in Long-Evans rats

Authors: *S. N. WATTS[1], A. CHAPMAN[2], M. MARCZAK[3], M. BARDI[4];
[1]Data Sci., Tufts Univ., Medford, MA; [2]Dept. of Physiol., Michigan State Univ., East Lansing, MI; [3]Eastern Virginia Med. Sch., Norfolk, VA; [4]Psychology and Behavioral Neurosci., Randolph-Macon Col., Ashland, VA

Abstract: Caloric restriction (CR) has been proposed as a potential solution for neuroinflammation associated with obesity and aging. Research has shown that long-term CR, without malnutrition, is effective in promoting longevity, weight loss, and reducing inflammatory factors in obese and elderly populations. However, there is a surprising lack of data on the short-term effects of CR in healthy adult models; especially in female-representing populations. Preliminary data from this study has shown that short-term CR in young adult Long-Evans rats can significantly decrease macrophage and inflammatory markers as well as significantly increase cytokine expression in the basolateral amygdala of both males and females. Young rats, 56 days old at the start of treatment, were randomly assigned to either standard diet

(SD) treatment or CR treatment. Rats in the CR treatment group received 30% less than the individual daily average intake with a custom food diet. The average weights of each group were compared on a weekly basis. Immunohistochemistry was used to measure Interleukin-6 (IL-6), Interleukin-2 (IL-2), Cluster of Differentiation-68 (CD68), Cluster of Differentiation-45 (CD45), Cluster of Differentiation-4 (CD4), Cluster of Differentiation-11b (CD11b), and Tumor Necrosis Factor-α (TNF- α) expression in the basolateral amygdala. Results demonstrated a significant increase in IL-6 and a significant decrease in CD68, CD4, and IL-2. We concluded that short-term CR restriction in young adult rats may be effective in reducing macrophages, but could have an adverse effect on the expression of both pro- and anti-inflammatory cytokines.

Disclosures: S.N. Watts: None. **A. Chapman:** None. **M. Marczak:** None. **M. Bardi:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.07/X1

Topic: G.08. Food and Water Intake and Energy Balance

Support: Purdue CEREBBRAL Pilot Grant

Title: Regulation of asprosin in the arcuate nucleus of the hypothalamus by nutritional state.

Authors: *E. MET HOXHA[1], E. BONANNO[1], C. MILLER[1], B. BACHMAN[2], K. KINZIG[1]; [1]Purdue Univ., West Lafayette, IN; [2]Duke Univ., Durham, NC

Abstract: Asprosin is a fasting-induced adipokine that crosses the blood-brain barrier and activates orexigenic agouti-related peptide (AgRP) neurons in the arcuate nucleus (ARC) of the hypothalamus, suggesting a role in appetite regulation. While fasting increases asprosin in the periphery and cerebrospinal fluid, it is unclear whether ARC asprosin expression changes in response to fasting. To test this, we assigned adult male and female Long Evans rats (n = 24; 6 per sex per condition) to either a sated or overnight fasted condition. Brains were collected and processed for immunohistochemical detection of asprosin in the ARC. Asprosin expression was quantified as the percentage of the total image area covered by asprosin-positive staining using image thresholding and automated analysis. We hypothesized that overnight fasting would increase asprosin levels in the ARC. A 2 x 2 ANOVA (sex x condition) revealed no significant main effects or interactions. However, preliminary findings suggest a potential increase in ARC asprosin expression in fasted animals compared to sated controls. To confirm this finding and expand on whether fasting reliably induces a peripheral increase in asprosin, ongoing work in our lab is increasing the sample size and measuring circulating plasma asprosin levels. Together, these data will provide insights into the mechanisms by which energy state modulates hypothalamic circuits involved in appetite and metabolism.

Disclosures: E. Met Hoxha: None. **E. Bonanno:** None. **C. Miller:** None. **B. Bachman:** None. **K. Kinzig:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.08/X2

Topic: G.08. Food and Water Intake and Energy Balance

Support: CNPq
FAPESP
PROPE UNESP

Title: Purinergic P1 receptor activation reduces pilocarpine-induced salivation

Authors: *P. M. DE PAULA[1,2], I. C. MORI[1], C. A. ANDRADE[1], L. A. DE LUCA[1], J. MENANI[1];
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Abstract: Intraperitoneal (ip) injection of pilocarpine (PILO, cholinergic muscarinic agonist) induces salivation and water intake. These effects are due to a direct action on the salivary glands and central nervous system (CNS). ATP (adenosine 5'-triphosphate) and its metabolite adenosine (ADN) are neurotransmitters that act peripherally and in the CNS activating purinergic P2 and P1 receptors respectively located in various regions including the brain and salivary glands. Preliminary results from our laboratory showed that ATP injected ip reduced PILO-induced salivation and water intake; suggest that purinergic P2 receptors participate in salivary secretion and drinking. The aim of the present study was to investigate the effects of ip injection of ADN (a natural agonist of purinergic P1 receptors) on salivation and water intake induced by PILO also administered ip. Male Holtzman rats (300-350 g, n=8-12) were used. The experimental protocols were approved by the Ethical Committee for Animal Care and Use - CEUA - from the Dentistry School of Araraquara, UNESP (Proc. CEUA nº 11/2019). A group of rats received ip injection of PILO (1 mg/kg of body weight) to induce salivation, in a salivation test, and water intake, in a drinking test. Ten minutes before, half of the group received an ip injection of ADN (50 mg/kg of body weight) and the other half received vehicle (dimethyl sulfoxide, DMSO, 50%). For the salivation test, rats were anesthetized with ketamine (100 mg/kg of body weight) ip, and saliva was collected using small cotton balls previously weighed inserted into the oral cavity of the rat. Previous ip injection of ADN (50 mg/kg of body weight) reduced the salivation induced by PILO ip (252 ± 29 mg/7 min, vs. DMSO + pilocarpine: 365 ± 32 mg/7 min, $p<0.05$). For the drinking test, water was offered in graduated burettes fitted with metal drinking spouts. The ip injections of ADN produced no change on PILO-induced water intake (2.6 ± 0.4 ml/60 min, vs. DMSO + PILO: 3.1 ± 0.4 ml/60 min). Different from what happens to P2 receptors, the results suggest that peripheral purinergic P1 receptors participate mainly in the control of salivary secretion induced by PILO.

Disclosures: P.M. De Paula: None. **I.C. Mori:** None. **C.A. Andrade:** None. **L.A. De Luca:** None. **J. Menani:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.09/X3

Topic: G.08. Food and Water Intake and Energy Balance

Support: Intramural Research Program
DK075021

Title: Receptor-mediated activation of $G_{12/13}$signaling in AGRP neurons regulates key metabolic functions

Authors: *D. HASPULA[1], Z. CUI[1], S. PITTALA[1], Y. CUI[1], J. AJWANI[2], O. GAVRILOVA[1], K. W. WILLIAMS[2], J. WESS[1];
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Abstract: Agouti-related peptide (AgRP) neurons of the arcuate nucleus (ARC) play a critical role in regulating appetite and energy homeostasis. Stimulation of these neurons leads to the release of neuropeptides, such as AGRP and neuropeptide Y, which strongly promote food intake. AGRP neurons express many G-protein coupled receptors, several of which activate G proteins of the $G_{12/13}$ family, besides other classes of heterotrimeric G proteins. The potential metabolic relevance of $G_{12/13}$ signaling in AGRP neurons remains unexplored. To address this question, we generated mice expressing a $G_{12/13}$-coupled designer receptor ($G_{12/13}$ DREADD; $G_{12/13}$D) specifically in AGRP neurons of the ARC (AGRP-$G_{12/13}$D mice). Additionally, we generated mice expressing a constitutively active version of Gα12 (CA-G12) specifically in ARC AGRP neurons (AGRP-CA-G12 mice) as well as mice lacking the α-subunits of $G_{12/13}$ in all AGRP neurons (AGRP-G12/13 KO mice). We administered deschloroclozapine (DCZ), a highly selective and pharmacologically inert DREADD agonist, via the drinking water for up to 8 weeks to $G_{12/13}$D mice and control littermates. Activation of G_{12} signaling in both AGRP-$G_{12/13}$D and AGRP-CA-G12 mice (not treated with DCZ) led to significant weight gain, increased adiposity, and impaired insulin resistance. These metabolic deficits were caused by increased food intake and decreased energy expenditure. Whole-cell patch clamp electrophysiological recordings revealed that $G_{12/13}$D activation in AGRP neurons caused ROCK-dependent neuronal depolarization. Finally, we found that the orexigenic effect of ghrelin was greatly diminished in AGRP-$G_{12/13}$ KO mice, indicating that efficient stimulation of food intake by ghrelin requires $G_{12/13}$ signaling. In vitro biochemical studies and electrophysiological experiments with AGRP neurons further confirmed that ghrelin-stimulated effects were dependent on $G_{12/13}$ signaling. Collectively, our data indicate that $G_{12/13}$ signaling in AGRP neurons plays a critical role in the maintenance of energy homeostasis. Clearly, our findings are of potential translational relevance. $G_{12/13}$-coupled receptors endogenously expressed by AGRP neurons may prove novel targets for therapeutic agents useful for the treatment of various metabolic disorders including obesity.

Disclosures: D. Haspula: None. **Z. Cui:** None. **S. Pittala:** None. **Y. Cui:** None. **J. Ajwani:** None. **O. Gavrilova:** None. **K.W. Williams:** None. **J. Wess:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.10/X4

Topic: G.08. Food and Water Intake and Energy Balance

Title: Estradiol mitigates the impact of an obesogenic diet on weight gain by altering taste function and the central processing of meal-related satiation signals

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Abstract: Diet-induced obesity (DIO) is studied primarily in male rodents, with a limited literature in females suggesting that they are less susceptible to DIO than males. While the inhibitory effect of estradiol (E2) on chow intake is well established, less is known about its ability to reduce intake of obesogenic diets. Here, we investigated how the loss of E2 in an OVX model affects caloric intake and weight gain in female rats maintained on chow or high fat diet (HFD). We also examined how the loss of E2 affects neuronal activity in the nucleus of the solitary tract (NTS), which processes meal-related satiation signals, and taste function as assayed by the number of fungiform papillae. In the current study, ovarian-intact (INT) and ovariectomized (OVX) rats with or without E2 replacement (OVX-veh, OVX-E2) were fed chow or 45% HFD. Food intake and body weight were measured daily for 4 weeks, after which hedonic eating was assessed via a “dessert” test in which rats were given 30-min access to chocolate Ensure immediately after consuming a satiating meal. Sucrose intake was then measured by a series of two-bottle preference tests (sucrose vs. water). Following this, rats were perfused 60 min after consuming a standardized meal (5g of HFD). Brain tissue was processed via IHC to quantify meal-stimulated cFos expression in the NTS, and tongues were stained to facilitate quantification of fungiform papillae. Cumulative 4-week weight gain was increased by HFD in ovarian intact rats (INT-HFD: 33.7 g vs. INT-chow: 19.1 g, a 23.6% increase), and, to a greater extent, by the loss of E2 in OVX rats (OVX-oil-chow: 63.7 g vs OVX-E2-chow: 26.3 g, a 42.2% increase). The loss of E2 also increased chocolate Ensure “dessert” intake in both chow- and HFD-fed rats (e.g., OVX-oil-HFD:19.6 kcal vs OVX-E2-HFD:11.5 kcal), and intake of 0.3M sucrose (e.g., OVX-oil-HFD: 118.1 g vs. OVX-E2-HFD: 98.8 g). Regardless of diet history, loss of E2 decreased meal stimulated cFos expression in the NTS (e.g., OVX-oil-HFD: 50.0 cells vs OVX-E2-HFD: 82.3 cells). Finally, the percentage of active fungiform papillae was decreased by the loss of E2 in chow- and HFD-fed rats (e.g., OVX-oil-HFD: 83.6% vs OVX-E2-HFD: 87.7%). This study highlights the critical role of E2 in the protection against DIO in female rats. Our findings demonstrate that loss of E2 promotes greater body weight dysregulation than the consumption of an obesogenic diet, an effect that may be mediated by

E2's ability to increase meal-related satiation signals and decrease hedonic eating through changes in taste bud number. Our findings are significant in that they offer insights that could inform strategies for addressing obesity in a sex-specific manner.

Disclosures: S.S. joyner: None. **D. Gaines:** None. **L.A. Eckel:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.11/X5

Topic: G.08. Food and Water Intake and Energy Balance

Support: NIDDK (DK134609)
HHMI
CZI
Dorris Neuroscience Scholarship

Title: Time-series modeling defines multidimensional animal states to uncover dynamic behavior-physiology interactions

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Abstract: Animals adapt to changing environments by adjusting their behaviors and internal states. These state changes shape neural and behavioral outputs. While behaviors and neural activities have been measured quantitatively with high dimensionality, temporal resolution, and accuracy, physiological states, which remain challenging to quantify, are typically vaguely characterized as binary conditions, such as hunger or satiety. The obscure definition of states limits our mechanistic and dynamic understanding of how organismal-level physiological and behavioral decisions are made. Here, we address this gap and advocate for quantitatively defining these states by analyzing real-time interactions between physiology and behavior. We leverage metabolic cages, also known as indirect calorimetry, which are commonly used in physiology research, to record multidimensional, fine-grained time-series physiological and behavioral variables, such as energy expenditure (EE), blood glucose, food intake, and movement. We develop a robust time-series analysis pipeline based on a time-lagged linear mixed model (TL-LMM) to quantify the temporal relationship between physiological and behavioral variables. Traditional analyses of metabolic cage data typically calculate the averages of those variables over extended periods, ranging from hours to weeks, thereby overlooking real-time correlations. In contrast, our model quantifies dynamic temporal relationships, and the fitted temporal filters capture how changes in one variable affect another over time. Next, we validate

the robustness and generalizability of our pipeline by characterizing baseline interactions under day-night cycles across three independent metabolic cage datasets. Furthermore, our pipeline explains how two energy-deficit conditions, cold exposure and fasting, divergently perturb baseline interactions, including the interaction between water intake and body temperature. Our pipeline also explores how anti-obesity drugs, like GLP-1 receptor agonists, disturb baseline physiology-behavior interactions. In addition, we demonstrate that short-term high-fat diets alter the interaction between EE and food intake. Together, our results provide a powerful time-series modeling pipeline for quantitatively redefining states by capturing the temporal interactions between physiology and behavior, enabling precise and mechanistic insights into metabolic phenotypes beyond traditional average-based analyses.

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Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.12/X6

Topic: G.08. Food and Water Intake and Energy Balance

Support: NIH R01DK141495

Title: A single dorsal vagal complex circuit mediates the aversive and anorectic responses to GLP1R agonists

Authors: *W. T. YACAWYCH[1,2], Y. WANG[1,4], S. HASSAN[5], F. SASS[1], M. DEVAUX[1], I. WU[1,2], J. BROWN[5], A. C. RUPP[1], A. SECHER[6], T. PERS[5], R. SEELEY[3], M. G. MYERS, Jr.[1,2], W. QIU[7,8];
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Abstract: GLP-1 receptor agonists (GLP1RAs) act via brainstem circuits to reduce feeding, yet also trigger aversive gastrointestinal events that limit treatment. While the physiologic systems that govern satiety and nausea are distinct, it remains unclear whether the aversive pharmacologic actions of GLP1RAs can be separated from their effects on weight loss. Thus, we sought to distinguish the physiologic and pharmacologic contributions of area postrema and *nucleus tractus solitarius Glp1r*-expressing neurons (AP^{Glp1r} and NTS^{Glp1r}neurons, respectively) in the control of long-term food intake, body weight, and aversion. Although silencing non-

aversive NTS^{Glp1r} neurons increased food intake and body weight, selectively restoring NTS *Glp1r* expression on an otherwise *Glp1r*-null background failed to enable long-term food intake or body weight suppression by GLP1RAs. Conversely, while silencing aversive AP^{Glp1r} neurons failed to alter energy balance, selective *Glp1r* expression in AP^{Glp1r} neurons restored the ability of GLP1RAs to promote aversion, suppress body weight, and inhibit fasting-activated arcuate nucleus neurons. Thus, AP^{Glp1r} neurons are crucial for both the anorectic and aversive responses to GLP1RAs, while NTS^{Glp1r} neurons mediate neither pharmacologic response.Because distinct *Glp1r*-expressing brainstem circuits exert physiologic control over satiety and nausea, it will be important to understand why non-aversive NTS^{Glp1r} satiety cells fail to mediate the pharmacologic weight loss response to GLP1RAs. We are now determining whether this failure results from inadequate *Glp1r*expression and/or insufficient GLP1RA access to the NTS^{Glp1r} neurons.

Disclosures: W.T. Yacawych: None. **Y. Wang:** None. **S. Hassan:** None. **F. Sass:** None. **M. deVaux:** None. **I. Wu:** None. **J. Brown:** None. **A.C. Rupp:** None. **A. Secher:** A. Employment/Salary (full or part-time): Novo Nordisk. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Novo Nordisk. **T. Pers:** None. **R. Seeley:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Novo Nordisk, Fractyl, Astra Zeneca, Congruence Therapeutics, Eli Lilly, Bullfrog AI, Glycsend Therapeutics, Amgen. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Calibrate, Rewind, Levator Therapeutics. F. Consulting Fees (e.g., advisory boards); Novo Nordisk, Eli Lilly, CinRx, Fractyl, Structure Therapeutics, Crinetics, Congruence Therapeutics. **M.G. Myers:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Novo Nordisk, Eli Lilly, AstraZeneca. F. Consulting Fees (e.g., advisory boards); Merck. **W. Qiu:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Topic: G.08. Food and Water Intake and Energy Balance

Support: NIH T32 DC-000044

Title: Semaglutide modulates the impact of diet on binge eating, hedonic eating, and neuronal activity in the nucleus of the solitary tract in female rats

Authors: *D. M. GAINES[1], L. A. ECKEL[2];
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Abstract: Semaglutide (SEMA), a glucagon-like peptide-1 (GLP-1) receptor agonist, is effective in decreasing food intake and promoting weight loss in individuals with obesity. Less is known, however, about SEMA's ability to attenuate binge eating in individuals with bulimic syndromes. While clinical studies suggest that SEMA decreases self-reported symptoms of binge eating, its ability to decrease caloric intake during episodes of binge eating is unknown. Here, we used a rat model of binge-like eating to investigate SEMA's effects on food intake, hedonic eating, and neuronal activity in the nucleus of the solitary tract (NTS), a brain area that processes meal-related satiation and taste signals. To induce binge eating, female rats were maintained on chow with intermittent access to 45% high fat diet (HFD) at 4-day intervals (INT group). Control groups were fed chow or chow and HFD (CHOW, HFD groups). Within these diet groups, rats received daily injections of SEMA (70 mg/kg) or vehicle throughout the study. After the binge-eating phase, hedonic eating was assessed via a "dessert" test in which rats were given 30-min access to chocolate Ensure immediately after consuming a satiating meal. Finally, rats were perfused 60 min after consuming a standardized meal (4 g of HFD) and brain tissue was processed via IHC to quantify cFos expression in the NTS. During the binge-eating phase, SEMA decreased cumulative food intake in INT and chow groups, but not in the HFD group. On "binge" days, INT-veh rats displayed binge-like eating during the first 30 min of the dark phase (35.3 kcal) in comparison to CHOW-veh and HFD-veh rats (4.0 and 4.4 kcal). SEMA suppressed this binge-like eating in INT rats (INT-SEMA: 27.6 kcal vs INT-veh: 35.3 kcal) but had no effect in the CHOW or HFD control groups. During the dessert test, SEMA decreased chocolate Ensure intake by ~40% in INT rats (INT-SEMA: 13.0 kcal vs. INT-VEH: 18.2 kcal) but had no effect in the CHOW or HFD control groups. Regardless of diet history, SEMA increased meal-stimulated cFos expression in the caudal NTS, which processes satiation signals arising from the gut (SEMA: 60.3 cells vs. VEH: 31.0 cells). Relative to chow-fed rats, a history of HFD intake decreased cFos expression in the rostral NTS, which processes taste-related information (INT: 22.6 cells and HFD: 17.5 cells vs CHOW: 38.1 cells). Our findings demonstrate that SEMA decreases caloric intake during episodes of binge eating, and that this action of SEMA may be mediated by its ability to modulate meal- and taste-stimulated activation of neurons in the NTS. We conclude that GLP-1 receptors offer a viable therapeutic target for treating binge eating in individuals with bulimic syndromes.

Disclosures: D.M. Gaines: None. **L.A. Eckel:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.14/X8

Topic: G.08. Food and Water Intake and Energy Balance

Title: The effect of food restriction on the estrus cycle in POMC-deficient mice

Authors: *B. MCHOES, Z. THOMPSON;
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Abstract: Expression of the pro-opiomelanocortin (POMC) gene in the hypothalamus leads to the production of several hormones, including α-melanocyte stimulating hormone (α-MSH) and β-endorphin (β-EP). α-MSH is a metabolic response hormone that helps to regulate food intake in response to energy needs. In humans, mutations in the POMC gene lead to reduced or no expression of the gene, and to excessive hunger and eventually obesity. We are studying a mouse model with a recessive mutation that results in little to no expression of the POMC gene in the hypothalamus. Like POMC-deficient humans, these mice exhibit hyperphagia, early-onset obesity and they also seem to be infertile. We are attempting to elucidate the cause of this infertility by limiting the food intake of the POMC-deficient mice to eliminate the obesity factor. Once the weight of the POMC-deficient mice are within normal range, we will track the estrus cycle in female mice. Tracking the estrus cycle can be used as a proxy for both blood estrogen levels and fertility. To track the estrus cycle, we obtain daily vaginal samples from the mice by flushing the vaginal canal with 0.9% saline solution. These samples are then pipetted onto a slide, and the number and types of cells present are estimated. Based on the cell characteristics of the sample, the mouse is determined to be in one of the four stages of the estrus cycle: proestrus, estrus, metestrus or diestrus. The cycle tracking data is compared to wild type mice as well as POMC-deficient mice with an unrestricted diet. These results will help us understand more about whether or not obesity is the primary cause of infertility in the POMC-deficient mouse model, providing a better understanding of the effects of POMC deficiency, and the relationship between obesity and infertility as well.

Disclosures: B. McHoes: None. **Z. Thompson:** None.

Poster

PSTR083: Integration of Peripheral Signals

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Program #/Poster #: PSTR083.15/X9

Topic: G.08. Food and Water Intake and Energy Balance

Support: Lewis College Foundation Award

Title: Neurobiological Insights from the Hippocampus and Prefrontal Cortex into the Effects of Wild Blueberry Supplementation Under High-Fat High-Sugar Dietary Conditions

Authors: *W. ELAMAD[1], S. VARGHESE[2], J. DANH[1], A. M. MABB[3], D. WANDERS[4], R. FERESIN[1];
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Abstract: Emerging evidence implicates Western-style diets rich in fat, salt, and sucrose (HFHSS) in disrupting neuroplasticity, astrocytic activity and cognitive performance. Anthocyanins, bioactive flavonoids abundant in blueberries (BB), possess antioxidant and neuromodulatory properties that may lead to neuroprotection. This study assessed the impact of

BB supplementation on hippocampal and prefrontal cortex-dependent behaviors, alongside region-specific neuroinflammatory and neuroplasticity biomarkers in male UMHET3 mice. Mice were acclimated to a low-fat low-sucrose (LFLSS) control diet or LFLSS + BB, then randomized to LFLSS, HFHSS, or HFHSS + BB. Spatial learning and memory were evaluated using the Barnes Maze task followed by a reversal phase. HFHSS-fed mice exhibited a trend toward increased latencies to locate the target hole ($p > 0.05$), without major impairments in reversal learning. Notably, BB-supplemented mice performed comparably to LFLSS controls. In the fear conditioning assessment, all groups demonstrated comparable learning during training. During contextual recall, HFHSS + BB mice showed a significant reduced freezing duration compared to LFLSS and a trend toward reduction compared to HFHSS ($p = 0.06$). During cue recall, no significant differences in freezing duration were observed across groups ($p = 0.13$), though HFHSS + BB mice exhibited a higher number of freezing episodes. At the molecular level, HFHSS significantly reduced hippocampal BDNF expression compared to LFLSS ($p = 0.05$), whereas this reduction was not observed in the BB-supplemented group ($p = 0.18$ vs. LFLSS), suggesting a potential protective effect of BB against diet-induced BDNF suppression. BB tended to attenuate HFHSS-induced astrocyte activation in the HPC, as evidenced by reduced GFAP ($p = 0.08$ vs HFHSS). Contrastingly, in the PFC, BB supplementation significantly elevated GFAP ($p = 0.02$ vs. LFLSS) and displayed a numerical increase in Claudin-1 ($p = 0.08$), suggesting compensatory astrocytic and blood-brain barrier remodeling. HFHSS reduced TLR4 and MyD88 expression ($p < 0.01$ vs. LFLSS), but there was no BB-mediated recovery ($p = 0.27$ vs. HFHSS). Despite a trend toward elevated pAkt/Akt in HFHSS ($p = 0.16$ vs. BB), reduced BDNF suggests a divergent mechanism of plasticity disruption, potentially independent of Akt and TLR signaling. Together, our findings may clarify the mechanisms by which BB could mitigate HFHSS-induced alterations in cognition and brain-region-specific molecular pathology.

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Poster

PSTR083: Integration of Peripheral Signals

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Topic: G.08. Food and Water Intake and Energy Balance

Support: UT Southwestern Medical Center Peter O'Donnell Jr. Brain Institute (OBI) Sprouts Program (to O.S.)
American Diabetes Association Postdoctoral Fellowship (#1-25-PDF-88 to O.S.)
National Institutes of Health (R01DK119341 to J.M.Z.)

Title: Temporal control of snacking and body weight by GHSR-expressing suprachiasmatic nucleus neurons

Authors: *O. SINGH[1,2], S. SHEYBANI DELOUI[1], S. KULKARNI[1], M. LYU[1], S. VARSHNEY[1], K. SHANKAR[1], D. GUPTA[1], L. LEON-MERCADO[1], A. W. BURSTEIN[1], J. ZIGMAN[1,2,3,4];
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Abstract: Food consumption impacts body weight differently depending on time-of-day. Here, we investigated whether suprachiasmatic nucleus (SCN) neurons responsive to the orexigenic hormone ghrelin temporally regulate eating and body weight in mice. Chemogenetic stimulation of GHSR (ghrelin receptor)-expressing SCN neurons during the mid-circadian rest phase - a period of low ghrelin resistance - increased food intake (by 144%, n=4). Repeated chemogenetic inhibition of these neurons during this same time-of-day reduced the corresponding, typically low level of food intake reminiscent of snacking (by 45%, n=4); it also reduced feed efficiency (by 87%, n=4), and body weight (by ~7%, n=4). These effects were not observed at other times-of-day. Chemogenetic stimulation of GHSR-expressing arcuate hypothalamic nucleus neurons increased food intake independently of time-of-day. GHSR-expressing SCN neurons represented subpopulations of six distinct SCN neuronal clusters, were predominantly GABAergic, and exhibited light-sensitive, time-of-day-dependent transcriptomic profiles. Thus, our study identifies GHSR-expressing SCN neurons as a neuronal population that regulates snacking, feed efficiency, and body weight in a mid-circadian rest phase-dependent manner.

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Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.17/X11

Topic: G.08. Food and Water Intake and Energy Balance

Support: F31 HL170784
AHA 935362
K99/R00 HL144923
T32GM144636

Title: Aldosterone-mediated sodium appetite requires mineralocorticoid receptor expression by hindbrain HSD2 neurons

Authors: *M. C. MCDONOUGH[1], A. KURALAY[3], T. BUTLER[3], M. SCHWAGER[1], S. GASPARINI[2], J. C. GEERLING[2], J. RESCH[1];

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Abstract: The hormone aldosterone is well known for its role in sodium retention but its ability to drive sodium intake is often overlooked. Further, primary hyperaldosteronism accounts for up to 20% of patients with treatment-resistant hypertension and is correlated with increased cardiovascular damage. Given that excess sodium intake may exacerbate hypertension and some primary aldosteronism patients report salt cravings, the mechanism by which aldosterone induces sodium appetite warrants further investigation. Notably, there is a uniquely aldosterone-sensitive neuron population in the hindbrain characterized by their expression of the mineralocorticoid receptor (MR), to which aldosterone binds, and 11β-hydroxysteroid dehydrogenase 2 (HSD2), which prevents glucocorticoid stimulation of MR. Moreover, activation of these "HSD2 neurons" using chemogenetic tools drives sodium appetite and their ablation eliminates sodium appetite during aldosterone treatment, demonstrating their necessity for aldosterone-induced sodium appetite. In order to investigate the importance of aldosterone signaling within HSD2 neurons, we used CRISPR-Cas9 to induce indel mutations within the MR gene (MR KO) specifically in HSD2 neurons. Our results show MR KO in HSD2 neurons significantly reduces sodium intake during sodium deficiency and completely abolishes sodium appetite induced by aldosterone (n=8 control, n=7 MR KO male mice). Given that MR regulates gene expression, we hypothesize that aldosterone-MR signaling induces sodium appetite by coordinating a transcriptional program promoting the excitability of HSD2 neurons. To elucidate the transcriptional targets of MR within HSD2 neurons that drive their increased activity, we performed single-nucleus RNA sequencing on HSD2 neurons after aldosterone treatment (n=16 male and 16 female mice treated with either vehicle or aldosterone). Importantly, transcriptional profiling of HSD2 neurons did not detect expression of epithelial sodium channel (ENaC) subunit genes, in agreement with previous studies, suggesting that renal and neuronal MR signaling have distinct mechanisms. Instead, aldosterone treatment induced expression of voltage-gated calcium channels (*Cacna1d and Cacna1i*), subunits of AMPA and kainate glutamate receptors (*Gria1* and *Grik4*, respectively), and synapse-associated genes (e.g., *Sv2c*, *Myrip*, and *Itpr1*), consistent with increased activity of HSD2 neurons. Future studies will aim to determine the molecular mechanisms driving aldosterone-mediated HSD2 neuron activation and its impact on sodium appetite.

Disclosures: M.C. McDonough: None. **A. Kuralay:** None. **T. Butler:** None. **M. Schwager:** None. **S. Gasparini:** None. **J.C. Geerling:** None. **J. Resch:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.18/X12

Topic: G.08. Food and Water Intake and Energy Balance

Title: Orexin neurons couple pupil-linked arousal to agrp hunger neurons, shaping adaptive eating and food value perception

Authors: *S. KOSAR, E. BRACEY, Y. DU, D. PELEG-RAIBSTEIN, D. BURDAKOV; ETH Zurich, Schwerzenbach, Switzerland

Abstract: Arousal and hunger are survival essentials, often dysregulated in human sleep-wake and eating disorders, but their interplay remains unclear. We show that their rapid fluctuations are linked, identify responsible neurons, and demonstrate the roles of this link in behavior, perception, and disease. First, using pupillometry as a direct metric of arousal, we found that self-paced pupil dilations coincide with inhibitions of hunger-causing Agrp neurons by using fiber optic photometry recording in freely moving mice. Pupil-linked arousal fluctuates second-by-second, closely correlating with dynamics of hypocretin/orexin (H/O) neurons, whose malfunctions are linked to multiple pathologies. It is currently thought that H/O neurons activate hunger-causing agouti-related peptide (AGRP) neurons, thus creating concurrent arousal and hunger. However, this theory struggles to explain increased eating in states of H/O neuron deficiency, such as narcolepsy.. Neuron-specific ablation and optostimulation revealed that pupil-tracking hypocretin/orexin (H/O) neurons are responsible for these inhibitions. Next, we compared Agrp neuron responses to different arousal-increasing sensory stimuli in mice with intact vs ablated H/O neurons. Arousing non-food stimuli distracted hungry mice from eating; this involved Agrp neuron inhibition, and required H/O neurons. With familiar food stimuli, we found that the feedforward inhibition of Agrp neurons during palatable food perception was also disrupted by H/O neuron ablation, producing Agrp hyperactivity. Finally, we used EEG-EMG recordings to track Agrp neurons during normal sleep and pathological sleep paralysis (cataplexy), which - together with unexplained overeating - is a key symptom of H/O neuron loss in humans. In H/O-deficient mice, we found that Agrp hyperactivation is time-locked to cataplexy and REM sleep. Together, these results reveal an unanticipated inverse link between volitionally-controllable arousal and hunger states, which relies on neurons that are prone to become defective during ageing and in many disorders.

Disclosures: S. kosar: None. **E. Bracey:** None. **Y. Du:** None. **D. Peleg-Raibstein:** None. **D. Burdakov:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.19/X13

Topic: G.08. Food and Water Intake and Energy Balance

Title: Assessment of Metabolic Dysfunction-Associated Steatotic Liver Disease in Patients with Diabetes in a Hospital in Mexico

Authors: L. D. MENDEZ-VAZQUEZ[1], J. D. CRUZ-CASTILLO[1], D. RUIZ[1], G. NOLASCO-ROSALES[1], E. RODRIGUEZ-SANCHEZ[3], E. E. SANTOS-HERNANDEZ[1], V. OLVERA HERNÁNDEZ[1], T. B. GONZÁLEZ-CASTRO[2], *I. E. JUAREZ-ROJOP[1];
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Abstract: Metabolic dysfunction-associated steatosis liver disease (MASLD) is characterized by steatosis in more than 5% of hepatocytes and impaired liver function. Besides, patients with diabetes have a 5.36-fold higher risk of developing MASLD. Given the clinical relevance of these complications, this study evaluated the presence of MASLD in patients with diabetes at Dr. Gustavo A. Rovirosa Pérez Hospital. We used the FIB-4 Score to identify liver dysfunction in this population. Sixty-eight patients with diabetes, of whom 20 had MASLD (11 women, 9 men), all had adequate glycemic control (HbA1c 7.0-7.5%; ADA 2025), and they presented obesity (BMI> 30) and elevated abdominal adiposity (waist-hip ratio > 0.9). MASLD patients had elevated triglycerides, urea, and creatinine, with decreased HDL and platelet counts ($p \leq 0.05$). Interestingly, women with MASLD had diminished total cholesterol, LDL, and platelet counts, as well as increased urea, creatinine, and alkaline phosphatase levels ($p \leq 0.05$), compared to women without MASLD. Men with MASLD showed a decrease in albumin and the albumin/globulin (A/G) ratio ($p \leq 0.05$). These findings indicate that women with diabetes and MASLD had an altered lipid profile and markers of liver damage, whereas men with MASLD had decreased liver proteins. Hepatic steatosis reflects a state of chronic metabolic dysfunction, affecting energy metabolism and cardiovascular risk in patients with diabetes. Furthermore, evidence suggests that obesity-related MASLD leads to low-grade cerebral tissue hypoxia, attributed to persistent inflammation and reduced cerebral blood flow. Additionally, some studies propose a possible association between MASLD and mild brain alterations or cognitive impairment. Therefore, we consider that early detection of MASLD using non-invasive tools, such as the FIB-4 Score, in patients with diabetes prevents the progression of complications, and that future studies should investigate the impact of MASLD on cognitive function.

Disclosures: L.D. Mendez-Vazquez: None. **J.D. Cruz-Castillo:** None. **D. Ruiz:** None. **G. Nolasco-Rosales:** None. **E. Rodriguez-Sanchez:** None. **E.E. Santos-Hernandez:** None. **V. Olvera Hernández:** None. **T.B. González-Castro:** None. **I.E. Juarez-Rojop:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.20/X14

Topic: G.08. Food and Water Intake and Energy Balance

Support: Wu Tsai Neurosciences Institute Grant

Title: Brain and body electrophysiological measures of interoception in patients with Avoidant/Restrictive Food Intake Disorder

Authors: *S. LIU[1], N. DATTA[2], A. S. PERLEY[3], J. LOCK[2], T. P. COLEMAN[3];
[1]Electrical Engin., [2]Psychiatry and Behavioral Sci., [3]Bioengineering, Stanford Univ., Stanford, CA

Abstract: Avoidant/restrictive food intake disorder (ARFID) is an eating disorder added to the DSM-5 in 2013. Like anorexia nervosa (AN), it is characterized by a restricted intake of calories. However, while restricted intake in AN is driven by a desire to change one's shape or weight, patients with ARFID restrict their intake due to: (1) sensory sensitivity (e.g., extreme dislike for certain textures or tastes of certain foods), (2) lack of interest in food or eating, (3) a fear of adverse consequences of eating (e.g., choking), or a combination of the three. This study aims to investigate and quantify if youth with ARFID have altered gastric interoception, or body cue processing. With the approval of the IRB at Stanford University, we obtained electrophysiological recordings and behavioral reports from 6 ARFID patients and 12 healthy control (HC) subjects aged 8-22 years old. Participants were asked to complete the Patient Assessment of Gastrointestinal Disorders-Symptom Severity Index (PAGI-SYM), a gastrointestinal symptom questionnaire, and a heartbeat counting task, a cardiac interoceptive behavioral task. Afterwards, simultaneous EEG and electrogastrography (EGG) were recorded before and after participants completed a two-stage water load test (WLT-II). Preliminary results from the PAGI-SYM scores indicate ARFID patients feel excessive fullness more quickly than HC, which can affect food intake. In addition, we found that while the two groups performed similarly on the heartbeat counting task, ARFID on average had a higher WLT-II index. Peripheral electrophysiological measurements showed that while HC demonstrated a statistically significant increase in EGG power post-drink compared to pre-drink, the change is not significant with the ARFID population. EEG measurements show that on average, alpha power in the parietal lobe-a region that in prior literature has been implicated in processing gastrointestinal sensations-in HC is higher in both pre- and post-drink than ARFID. In addition, the drop in alpha power in this region is greater in HC. Overall, these preliminary findings suggest that ARFID patients may have heightened gastric sensitivity, or perceived satiety. As a result, this can lead to perceptions of fullness even when there has not been significant gastric distension (e.g., prematurely experiencing fullness when drinking water) resulting in less dramatic changes in stomach and brain electrophysiological measures in ARFID. This suggests that objective physiological profiles of gastric sensation and its enteric and central nervous system manifestations can provide a window into the mechanisms of this newly defined eating disorder.

Disclosures: S. Liu: None. **N. Datta:** None. **A.S. Perley:** None. **J. Lock:** None. **T.P. Coleman:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.21/X15

Topic: G.08. Food and Water Intake and Energy Balance

Support: Ketterer-Vorwald Neurosciences Interdisciplinary Graduate Fellowship

Title: Multi-modal physiologic assessment of the vagal cholinergic pathway during the cephalic phase of digestion

Authors: *Y. M. KEHNEMOUYI, T. P. COLEMAN;
Bioengineering, Stanford Univ., Stanford, CA

Abstract: It is increasingly recognized that many neurologic, neurodegenerative, and psychiatric illnesses exhibit dysbiosis between the central (brain) and enteric (gut) nervous systems as part of their pathophysiology. The vagus nerve, an important linkage between these two nervous systems, supports many bodily functions including digestion, cognition, and sleep. Although the majority of vagal signaling is afferent, the efferent vagal cholinergic pathway is implicated in many gut-brain disorders. This pathway plays a primary role during the cephalic phase of digestion. Within this context, presentation of an exteroceptive food-related sensory input generates anticipatory vagal efferent signaling from the brainstem to the stomach to begin digestive processes.Typical studies of the cephalic phase indirectly assess the integrity of the vagal cholinergic pathway by measuring gastric acid secretion or pancreatic polypeptide response during sham feeding. Multiple drawbacks exist, including: i) sham feeding limits the type of sensory input to taste alone; ii) the measurements are invasive; iii) the measures are only gastric readouts and thus do not directly probe dysfunction of the vagal cholinergic pathway.Here, we simultaneously measure human brain, stomach, and glossopharyngeal activity non-invasively with electroencephalography (EEG), electrogastrography (EGG), and throat electromyography (EMG) respectively during the cephalic phase. 15 college-aged healthy individuals (9 female, 6 male) were consented and identified a personalized meal that was appetitive. During the fasted state, each subject first completed a 15-minute seated baseline recording. Then, to elicit cephalic stimulation for another 15 minutes, one observed a video of meal preparation (auditory stimuli) and experienced the aroma of their meal (olfactory stimuli). After the video, the personalized meal is placed in front of them (visual stimuli).EEG was filtered to alpha band (8-12 Hz), EGG to 0.03-0.07 Hz, and swallow EMG to below 0.5Hz. We extracted EEG alpha power, EGG power, EEG alpha band phase lock value (PLV), and the interswallow interval (ISI) in each 15-minute period. We observed higher average EGG power ($p<0.05$), lower average ISI ($p<0.001$), decreased average EEG alpha power in all five cortical lobes ($p<0.05$), and global decreased EEG PLV ($p<0.01$) during the cephalic phase as compared to baseline. These findings provide novel, non-invasive, and multimodal neurophysiological markers to track the cephalic phase of digestion, enabling assessment of vagus nerve integrity to improve the diagnosis of gut-brain disorders.

Disclosures: Y.M. Kehnemouyi: None. **T.P. Coleman:** None.

Poster

PSTR083: Integration of Peripheral Signals

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR083.22/X16

Topic: D.01. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH K99DK139407
AHA 23POST1030352

Title: A cold-responsive neural circuit orchestrates systemic immune response

Authors: *H. LIU[1], Y. XU[2];
[1]Pediatrics, [2]Baylor Col. of Med., Houston, TX

Abstract: A tightly regulated immune response is critical for survival and fitness, and accumulating evidence highlights bidirectional communication between the immune and central nervous systems. Although cold exposure has been shown to modulate immunity, the neural substrates involved remain undefined. Here, we show that that cold exposure elevates circulating corticosterone and norepinephrine levels, which in turn suppress lipopolysaccharide (LPS)-induced inflammation in mice. Using c-Fos mapping and in vivo fiber photometry, we identified that cold exposure activates tyrosine hydroxylase (TH)-expressing neurons in the locus coeruleus (LC). Chemogenetic stimulation of LC^{TH} neurons raises serum corticosterone and norepinephrine, and markedly reduces LPS-induced inflammation. We further found that LC^{TH} neurons send dense projections to the paraventricular nucleus of the hypothalamus (PVH), and activation of LC^{TH} neurons robustly excites PVH corticotropin-releasing hormone (CRH)-expressing neurons. Conversely, silencing LC^{TH} neurons prevents cold-induced PVH^{CRH} activation, lowers corticosterone and norepinephrine levels, and diminishes the anti-inflammatory effects of cold exposure. Collectively, our findings reveal a cold-responsive LC^{TH} → PVH^{CRH} circuit that orchestrates neuroendocrine release to mitigate systemic inflammation.

Disclosures: H. Liu: None. **Y. Xu:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.01/Y1

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIH Grant R21MH133055

Title: Effects of intracranial stimulation on regulation of emotional threat response depend on anatomical location and tissue type in humans

Authors: *R. FONTENOT[1], M. OATES[1], A. BOLARAM[1], R. CHATFIELD[1], B. COX[1], A. FOBIAN[1], D. C. KNIGHT[1], J. F. MAGNOTTI[2], J. SZAFLARSKI[1], A. M. GOODMAN[1]; [1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Pennsylvania, Downingtown, PA

Abstract: Aberrant fronto-limbic network function may be a crucial mechanism underlying pathological disruptions in healthy emotion regulation. Causal brain mapping assessments can increase rigor in support of this model and translate to new treatment avenues. Thus, the current study assessed whether disruptive stimulation delivered to an a priori medial prefrontal cortex (mPFC, MNI: x=-4, y=40, z=25) region would modulate anticipatory regulation of autonomic responses to a threat. Participants (n=13) completed an auditory Pavlovian fear conditioning task during stereoelectroencephalography (sEEG) monitoring for refractory epilepsy, with electrode placements determined by clinical planning. Auditory threats (static) were presented alone (unpredictable) or with a warning tone (predictable) during skin conductance response (SCR) recording. Regulation of emotional responses to threat were demonstrated by greater SCR to unpredictable than to predictable threats [t=4.2, p<0.01]. After this association was formed, 2s pulse trains (20 Hz, 2 mA) were delivered during warning tones for predictable threats, however, no overall effect of stimulation on SCR was observed [sham vs stimulation; t=0.4, p=0.7]. We evaluated post-hoc anatomical heterogeneity of stimulation sites by comparing stimulation effects on SCR (mean difference) based on two factors: (1) tissue type (white vs. grey matter, determined by ICBM 2009c tissue probability maps) and (2) the site's relative anatomical position (MNI xyz coordinates). The results of these assessments yielded a significant relationship between inferior to superior z-values [r_s=0.7, p<0.05], but no overall difference between tissue types [t=0.5, p=0.6]. For mPFC grey matter, there was a significant relationship between stimulation effect on SCR and z-values [r_s= 0.8, p<0.05]; however, there was no relationship present between SCR and anatomical position for mPFC white matter sites [r_s= 0.1, p= 0.9]. In line canonical functional connectivity (FC) network models, anterior (default mode and limbic networks) differs from dorsal mPFC regions (salience and control networks) that may each underly opposing autonomic regulation processes. Thus, follow-up exploratory findings suggest that disruptive mPFC stimulation in white matter and ventral grey matter may inhibit bottom-up threat processing, decreasing threat responses. Alternatively, dorsal mPFC grey matter stimulation may inhibit regulatory processing, increasing threat responses. Future analyses using single pulse stimulation recordings will create individualized FC maps for each stimulation site to more precisely identify network connectivity.

Disclosures: R. Fontenot: None. **M. Oates:** None. **A. Bolaram:** None. **R. Chatfield:** None. **B. Cox:** None. **A. Fobian:** None. **D.C. Knight:** None. **J.F. Magnotti:** None. **J. Szaflarski:** None. **A.M. Goodman:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.02/Y2

Topic: H.01. Fear and Aversive Learning and Memory

Support: 2P50MH096889
1IK6BX006186

Title: Contribution of the paraventricular nucleus of the thalamus to remote fear learning in humans

Authors: C. HAYASHI[1], J. ROSS[2], G. A. CASTILLO[3], *V. B. RISBROUGH[4], D. M. STOUT[3];
[1]Univ. of California at San Diego, San Diego, CA; [2]UCSD Dept. of Psychiatry, San Diego, CA; [3]Psychiatry, [4]Univ. of California San Diego, San Diego, CA

Abstract: Post-traumatic stress disorder (PTSD) is characterized as a 'disorder of threat memory,' but the neural mechanisms subserving long-term threat memory are poorly understood. In animals, the paraventricular nucleus of the thalamus (PVT) plays a critical role in long-term threat memory (~7 Days) but not immediate threat learning (24hrs). Human threat learning paradigms typically examine threat memory within 24-48 hours, which may not adequately assess a "long-term" threat memory. The current study assessed behavioral, psychophysiological, and neural correlates of long-term threat memory in humans. Adult participants underwent threat acquisition on Day 1 (n=33). Then, 7-10 days later, participants returned for a threat memory recall task (n=31). Threat expectancy ratings, electrodermal activity, and functional magnetic resonance imaging (fMRI) were collected at both sessions. On Day 1, participants learned to discriminate between CS+ and CS- as measured by both expectancy ratings and cued skin conductance responses (SCR). At Day 7, there was significant threat recall for US expectancy ratings which diminished over the session due to extinction learning, but no threat recall as measured by SCR. For the PVT, there was a significant difference between the CS+ and CS- BOLD activity on Day 7, but not on Day 1. PVT connectivity changes from Day 1 to Day 7 will also be presented. The results suggest that conditioned threat responses developed during fear acquisition may diminish over time, but explicit episodic threat memory recall remains intact. We find preliminary support of the PVT plays a stronger role in long-term threat memory compared to threat learning. Future studies are needed to determine if this circuit is disrupted in PTSD, a disorder characterized but alterations in long-term threat expression.

Disclosures: C. Hayashi: None. **J. Ross:** None. **G.A. Castillo:** None. **V.B. Risbrough:** None. **D.M. Stout:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.03/Y3

Topic: H.01. Fear and Aversive Learning and Memory

Support: NSTC 113-2320-B-007 -005 -MY3 to CHC

Title: Examining the Neural Circuits underlying the Acquisition and Retrieval of Trace Fear Memory: The Role of the Perirhinal Cortex

Authors: *L.-H. CHEN[1], C.-H. CHANG[2];
[1]Inst. of Systems Neurosci., Natl. Tsing Hua Univ., Hsinchu, Taiwan; [2]Inst. of Systems Neurosci., Natl. Tsing Hua Univ., Hsinchu (city), Taiwan

Abstract: Fear conditioning serves as a tool to study the encoding and retrieval of fear memory. In trace fear conditioning (TFC), which has a temporal gap separating the conditioned stimulus (CS) and the unconditioned stimulus (US), the hippocampus (HPC) and the medial prefrontal cortex (mPFC) are critical for the modulation of the fear memory. As a hub linking the HPC and the mPFC, the perirhinal cortex (PRC) also contributed to TFC memory. Previous work from our lab has shown that the PRC is essential for both the acquisition and retrieval of TFC memory. However, the upstream inputs to the PRC involved in these processes remain unclear. In this study, we used a two-day TFC protocol with male Long Evans rats (6- to 8-week-old) to address this question. On conditioning day (Day 1), the animals were placed into conditioning chambers (Context A) for a 3-minute baseline (BL), followed by a 10-trial session of trace fear conditioning (the CS, 20-second tone, 85 dB, 2 kHz; the US, 2-second footshock, 1.0 mA; 240-second inter-trial interval [ITI]). On retrieval test day (Day 2), they were placed into a novel context (Context B) for a 3-minute BL, followed by three 20-second presentations of the CS, each separated by a 60-second ITI. The animals were divided into three groups based on their training procedures on Day 1: TRACE (trace interval of 30 seconds), UNPAIRED (random trace interval ranging from 100 to 120 seconds), and NoCOND (CS only). Within each group, half of the animals were euthanized after conditioning to assess memory acquisition, and the remaining half were euthanized after the retrieval test to examine memory retrieval. To identify activated neurons projecting to PRC, retrograde tracer Fluorogold (FG) was injected into the PRC before the behavioral procedures, and c-Fos expression in FG-labeled neurons was quantified postmortem. Our results show that both the UNPAIRED and TRACE groups showed equivalent increases in fear during conditioning. However, the TRACE group demonstrated higher cue-induced fear during retrieval, suggesting that the CS acquired the predictive value under trace procedure. Neuronal activation levels of key PRC-afferent regions from the HPC and the mPFC, including the dorsal CA1 (dCA1), ventral CA1 (vCA1), dorsal subiculum (dSub), ventral subiculum (vSub), prelimbic cortex (PL), and infralimbic cortex (IL), are being quantified and will be compared among the three groups to access the pathway-specific engagement of memory acquisition or retrieval. These results will help clarify the circuit-level mechanisms by which the PRC integrates temporally discontinuous information in fear memory.
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Disclosures: L. Chen: None. **C. Chang:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR084.04/Y4

Topic: H.01. Fear and Aversive Learning and Memory

Support: Swiss National Science Foundation Grant 310030_204587/1

Title: Direct pathway neurons in the auditory tail striatum induce movement arrest and their activity is sufficient for the behavioral expression of fear memories

Authors: *E. L. J. MOMBELLI, O. KOCHUBEY, R. SCHNEGGENBURGER;
Lab. of Synaptic Mechanisms, EPFL, Brain Mind Inst., Lausanne, Switzerland

Abstract: Fear learning allows the identification of threatening situations, a learning process instrumental for survival; moreover, a dys-regulation of neuronal fear circuits is at the root of anxiety - and post-traumatic disorders. Fear learning has been linked to the activity of the lateral and central amygdala; nevertheless, emerging evidence suggests a role of the tail striatum (TS) in the acquisition and expression of auditory-cued fear memories (Kintscher et al., 2023; Chen et al., 2023; Zafiri et al., 2025). However, only few studies have addressed the role of specific TS neuron populations, like 'direct' or 'indirect' pathway neurons, in fear learning (Kintscher et al., 2023). Furthermore, previous studies on the role of D1R direct pathway neurons might be limited by Cre mouse lines in which Cre expression does not faithfully recapitulate endogenous D1R expression in the TS. Because D1R neurons are known to co-express the Tac1 gene, we here characterized a $Tac1^{Cre}$ mouse (Harris et al., 2014) for studies of direct pathway neurons in fear learning. Using $Tac1^{Cre}$ mice crossed with a tdTomato reporter line (Ai9), we found that Cre expression faithfully overlaps with D1R expression throughout the entire TS. Moreover, $Tac1^{Cre}$ neurons in the TS (TS-Tac1+ neurons) are enriched in a medial band of the ventral TS, as shown before for D1R expression (Miyamoto et al., 2019). TS-Tac1+ neurons receive presynaptic inputs from the auditory thalamus and auditory cortex as shown by rabies-mediated trans-synaptic labeling. We next employed $Tac1^{Cre}$ mice as a suitable tool to study the role of direct pathway neurons in the TS in fear learning. Optogenetic stimulation of TS-Tac1+ neurons after Cre-dependent expression of Chronos (2 ms pulses at 25Hz for 30s; 10 mW) significantly reduced locomotion, coupled with horizontal, sweeping head movements, whereas in control mice expressing eGFP, blue light application was ineffective. Correspondingly, when mice were trained with weak footshocks in fear learning experiments (0.4 mA), optogenetic stimulation of TS-Tac1+ neurons was sufficient to bring CS-induced freezing back to normal levels. These findings with a newly established Cre mouse line show that the activity of direct pathway neurons in the TS reduces locomotion, and thus, that the normal function of striatal direct pathway neurons to favor movement is inverted in the tail striatum. Future work will investigate whether plasticity in TS-Tac1+ neurons is involved in the formation, and expression of an auditory-cued fear memory.

Disclosures: E.L.J. Mombelli: None. **O. Kochubey:** None. **R. Schneggenburger:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

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Program #/Poster #: PSTR084.05/Y5

Topic: H.01. Fear and Aversive Learning and Memory

Support: National Institutes of Health (NIH) grant 1R21NS10309
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MnDrive Data Science Initiative

Title: Characterizing the Effects of Fear Conditioning and Extinction in Mesoscale Cortical Dynamics of Mice

Authors: *M. R. LEHMAN[1,2], K. SAXENA[3], D. A. SURINACH[4], S. B. KODANDARAMAIAH[5];
[2]Biomed. Engin., [3]Mechanical Engin., [1]Univ. of Minnesota, Minneapolis, MN; [4]Neural Engin., Objective Biotech., Roseville, MN; [5]MECHANICAL ENGINEERING, Univ. Of Minnesota, Twin Cities, Minneapolis, MN

Abstract: The dorsal cerebral cortex contains many subregions which are implicated in sensory processing and the generation of motor plans and actions. These regions thus play a crucial role in animal behavior through coordination with other cortical and subcortical brain regions. To this end, the brain is capable of generating internal states, such as fear, that drastically change expected behavioral outcomes and wide-field brain activity. In this study, we show how fear-conditioning and extinction alters mesoscale spatiotemporal activity in the cerebral cortex. We used a miniaturized head-mounted microscope to image pan-cortical calcium activity in GCaMP6f transgenic mice undergoing Pavlovian fear-conditioning. On the first testing day, an experimental group of mice (n=8) was habituated to a 5kHz auditory tone, serving as the conditioned stimulus (CS), and subsequently subjected to five coterminous pairings of this tone with a 0.6mA 0.5s footshock, serving as an unconditioned stimulus (US). A control group (n=5) received exposure to the same CS and US in a pseudorandom unpaired fashion, so as not to induce CS-US association. On a second and third testing day, both the control and experimental group were exposed to ten CS presentations to test for fear and extinction learning. Mouse behaviors were manually scored for instances of freezing among both groups. Imaged brain data were input to a machine learning model that performs dimensionality reduction to decompose mesoscale calcium activity into most commonly expressed patterns of cortical activation or “temporary cortical states”. We use a simple probability model to stereotype sequences of temporary states that occur during freezing behaviors. In our conditioned group, we found two primary changes. First, paired CS-US mice exhibited a posterior-cortex activation dominated temporary state prior to the onset of a freezing event. Conversely, unpaired CS-US mice did not express any stereotyped temporary states prior to freezing. Second, cortical activity in paired CS-US mice progresses from posterior to medial to anterior activity over increasingly longer timescales during extinction, compared to unpaired CS-US mcie which exhibit the same sequences over the same timescales across all days. These results indicate dynamic changes to cortical calcium dynamics induced by fear conditioning.

Disclosures: M.R. Lehman: None. **K. Saxena:** None. **D.A. Surinach:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Objective Biotechnology. **S.B. Kodandaramaiah:** E. Ownership

Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Objective Biotechnology.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.06/Y6

Topic: H.01. Fear and Aversive Learning and Memory

Title: The role of the infralimbic cortex in mediating sex differences in contextual fear expression

Authors: K. VAZQUEZ, **T. STUART**, *R. G. PARSONS;
Stony Brook Univ., Stony Brook, NY

Abstract: Prior studies from our laboratory and others have reported that females exhibit reduced expression of freezing behavior when memory for contextual fear conditioning is tested. In more recent studies we examined neural activity associated with the expression of contextual fear in males and females using EGR1 immunofluorescence. We were interested in measuring neural activity in the prelimbic and infralimbic cortex, given their well-characterized involvement in regulating the expression of fear. We found that males exhibited an increase in EGR1 expression in the prelimbic cortex, whereas females did not. Conversely, females showed higher levels of EGR1 expression in the infralimbic cortex following testing when compared to males. The latter finding suggests that the low levels of contextual fear in females may be the result of increased activity of the infralimbic cortex. Here, we tested this hypothesis by using chemogenetics to inhibit excitatory neurons in the infralimbic cortex during the expression of contextual fear in male and female rats. Rats were surgically infused with a virus expressing the inhibitory chemogenetic construct hM4Di or a control virus and several weeks later were exposed to a contextual fear conditioning procedure. Shortly before an initial test session, rats were given IP injections of the chemogenetic agonist deschloroclozapine. Preliminary results indicate that inhibiting neural activity in the infralimbic cortex increased the expression of contextual fear in females, but not males. Freezing behavior to a novel context was not affected by chemogenetic inhibition of the infralimbic cortex in either females or males. Experiments are ongoing to determine how varying the timing of inhibition affects the expression of contextual fear.

Disclosures: K. Vazquez: None. **T. Stuart:** None. **R.G. Parsons:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.07/Y7

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIH Grant R21 MH121772

Title: Development and Application of a DeepLabCut Model for Startle Behavior

Authors: *Z. ANDERSON[1], R. G. PARSONS[2];
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Abstract: Prior studies from our laboratory, particularly those that have examined sex differences, have produced inconsistent results when comparing experiments in which freezing behavior was measured versus those that measured fear-potentiated startle These inconsistencies may stem from a variety of methodological factors, including parametric differences with the stimuli used to condition the animals and differences in the size of the experimental apparatuses. . Traditionally, startle behavior in rodents is measured using piezoelectric sensors or load cells, which require small chambers to accurately detect changes in Force. Freezing behavior can be difficult to measure in small enclosures, so larger chambers are typically used. To address this issue, we are developing a methodology using DeepLabCut, a pose estimation model, to create a model that will allow us to measure fear-potentiated startle in freezing chambers. Using the SuperAnimal Zoo model as a base, we will train on randomly selected, manually annotated frames captured from rodents in both freezing and startle chamber environments. Following initial training, once the confidence scores for prediction accuracy are above 85%, we will test the model's generalizability using novel videos not included in the training dataset. To analyze the startle behavior, reliable movement patterns and their corresponding body parts—specifically ear recoil, spinal arching, and tail extension—will be used to measure the startle response. Our recording protocol captures 200ms pre- and post-stimulus onset across multiple sound intensities (95dB, 105dB, and 115dB), with results analyzing the velocities to demonstrate amplitude differences across intensities. We will validate the model across multiple sound intensities, ensuring that these corresponding startle responses are consistent with previously established parameters. Additionally, we will conduct cross-chamber validation to ensure measurement reliability across startle and freezing chambers. This standardized approach will then be used to determine if the inconsistencies we observe between fear-potentiated startle and freezing behavior persist. In the future, this methodology provides a basis for investigating additional behavioral differences in both freezing and startle contexts.

Disclosures: Z. Anderson: None. **R.G. Parsons:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.08/Y8

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIGMS grant P20GM103446
NIGMS grant 1P20GM103653
University of Delaware Frontiers grant

Title: Using modular analysis and IEG expression to measure traumatic stress-induced changes in the default mode network

Authors: *S. LUNN[1], N. MOHAMMADMIRZAEI[7], P. P. KULKARNI[8], X. CAI[9], M. BIDDLE[2], K. MARVEL[3], A. SIPOS[4], E. LOPES[5], D. K. KNOX[6];
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Abstract: Disruptions in functional connectivity within the default mode network (DMN) have been consistently implicated in post traumatic stress disorder (PTSD), but it remains unclear exactly how disruptions in DMN connectivity lead to specific PTSD symptoms. Recent work we have conducted shows that DMN connectivity is altered in a rat model of PTSD (single prolonged stress (SPS)), and this disruption could be due to lowered connectivity involving the dorsomedial prefrontal cortex, retrosplenial cortex (RSC), and dorsal hippocampus. To further understand the impact of SPS on DMN connectivity we used a modular analysis, which allows for the examination of SPS on positive and negative connectivity within the DMN. Animals were scanned initially in a 9.5T Brucker magnet using in vivo MRI techniques to record resting-state functional connectivity, exposed to SPS or control stress, and then scanned a second time after SPS. We used t-test to define the rat-DMN as a module comprised of nodes and edges. Prior to stress, the anterior cingulate cortex (ACC) and RSC within the rat-DMN had the most edges, which is consistent with prior reports that defines the ACC and RSC as the anterior (ACC) and posterior (RSC) poles of the rat-DMN. This structure changed in control rats when animals were scanned a second time. There was an overall decrease in ACC edges, and in particular, negative edges between the ACC and dorsal CA1 (dCA1); ACC and RSC; and ACC and orbitofrontal cortex (OFC). This change in ACC connectivity was not observed in SPS rats. DMN connectivity decreases when an organism engages in a specific task, but it is difficult to implement in vivo MRI in behaving animals. To approximate DMN connectivity during emotional learning and memory, we generated a database of immediate early gene (IEG) expression throughout emotional centers in the brain at baseline (home colony), fear conditioning, extinction training, and extinction testing. We then used regression models to approximate connectivity among the nodes identified in our modular analysis (i.e. ACC, RSC, OFC, and dCA1). Analysis is ongoing and data from this final analysis will be presented at the SFN conference.

Disclosures: S. Lunn: None. **N. Mohammadmirzaei:** None. **P.P. Kulkarni:** None. **X. Cai:** None. **M. Biddle:** None. **K. Marvel:** None. **A. Sipos:** None. **E. Lopes:** None. **D.K. Knox:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.09/Y9

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIGMS grant P20GM103446
NIGMS grant 1P20GM103653(2,3)

Title: Ventral hippocampus inhibition leads to deficits in extinction of inhibitory avoidance

Authors: *D. K. KNOX[1], S. LUNN[3], N. MOHAMMADMIRZAEI[1], A. SIPOS[2], E. LOPES[2]; [2]Psychological and Brain Sci., [1]Univ. of Delaware, Newark, DE; [3]Dept. of Psychological and Brain Sciences, Univ. of Delaware, Newark, DE

Abstract: Post traumatic stress disorder (PTSD) is more prevalent in women than men, but mechanisms that facilitate this sex difference are not well understood. Previous research we have conducted suggests that midline thalamic-ventral hippocampal circuits may facilitate sex-differences in traumatic stress reactivity. To explore this possibility we designed experiments where we planned on inhibiting the ventral hippocampus (vHipp) during traumatic stress to determine if this prevented the impact of traumatic stress on fear extinction. Surprisingly we observed that inhibition of the ventral hippocampus (using a chemogenetic approach) in non-stressed rats disrupted extinction retention of inhibitory/passive avoidance in female rats. Using in vivo MRI techniques and a 9.5T Brucker scanner, we measured the volume of the hippocampus and resting state functional connectivity within emotional circuits prior to and after vHipp inhibition. Analysis is ongoing and data from these analyses will be presented at the SFN conference. However, preliminary results suggest that vHipp inhibition prevents adult expansion of the dorsal dentate gyrus. Lastly we describe a non-invasive technique to measure estrous cycle in female rats and determine if estrous cycle modulates any effects observed with vHipp inhibition.

Disclosures: D.K. Knox: None. **S. Lunn:** None. **N. Mohammadmirzaei:** None. **A. Sipos:** None. **E. Lopes:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR084.10/Y10

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Funding
P30 DA048736 (L.S.Z.)

Title: Understanding the role of CCK/CB1R expressing Gabaergic interneurons in the basolateral amygdala during fear extinction

Authors: *O. GUNDUZ CINAR[1], N. CROW[1], M. XIA[1], E. VAN LEER[1], S. PELAYO[1], L. S. ZWEIFEL[2], N. HAJOS[3], A. HOLMES[1];
[1]NIH, Natl. Inst. on Alcohol Abuse & Alcohol NIAAA, Rockville, MD; [2]Pharmacol., Univ. of Washington, Seattle, WA; [3]Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN

Abstract: Previously, we demonstrated the importance of cortical inputs into the basolateral amygdala (BLA) for extinction learning and showed that augmented endocannabinoids signal through their presynaptic receptors to modulate synaptic plasticity during extinction learning (Gunduz-Cinar et al 2023). However, the precise contribution of increased endocannabinoids on the CB1Rs expressing BLA cholecystokinin (CCK) interneurons are still not known. Our previous attempt to manipulate these GABAergic interneurons using the CCK-Cre-Dlx-FLpo double transgenic mice appeared to be not specific to the CB1Rs that are abundant on just the CCK interneurons, but also a variety of other cells (Rovira-Estaban et al 2019). In this study, using the intrsect viral strategy we explored the role of calcium binding protein of CCK/CB1Rs (NECAB 2) (Miczan et al 2021) that showed selectivity in hippocampus. Using viral promoters, we characterized their molecular expression in BLA and their behavioral roles during fear extinction. Profiling of Necab2 as a promoter for BLA CCK/CB1R-interneurons showed expression primarily on the GABAergic neurons but with lack of specificity evidenced by co-expression on NPY, VIP and PV INs. Moreover, CB1Rs were not abundantly expressed on Necab2 expressing interneurons. Understanding the precise role of the CB1R expressing interneurons will provide us information on input neuron modulation during fear and extinction learning in BLA. The results of this study will guide us in the development of better therapeutic strategies for anxiety and stress related disorders.

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Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.11/Y11

Topic: H.01. Fear and Aversive Learning and Memory

Support: R01MH117852

Title: Parvalbumin-positive interneurons in the basolateral amygdala mediate the immediate extinction deficit

Authors: *S. PLAS[1], K. CRAYTON[1], L. MATTANA FERST[1], A. ARSENYEVA[1], S. MAREN[2];
[1]The Univ. of Illinois at Urbana Champaign, Champaign, IL; [2]Psychology, Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Stress-induced hyperexcitability of basolateral amygdala (BLA) principal neurons is associated with impaired extinction learning, including the immediate extinction deficit (IED). BLA hyperexcitability is reduced by systemic administration of propranolol (PROP), a B-adrenergic antagonist, and PROP attenuates the IED. We hypothesize that these effects are mediated by parvalbumin-containing (PV+) inhibitory interneurons in the BLA. To test this, we performed two experiments. In Experiment 1, we examined whether optogenetically stimulating BLA PV+ interneurons during an immediate extinction procedure would prevent the IED. We expressed AAV-DIO-ChR2-mCherry in the BLA of male and female PV-Cre rats, as well as wild-type controls. Optostimulation of PV+ neurons during the conditioned stimulus did not affect freezing during the immediate extinction session, but did reduce freezing during retrieval testing in PV-Cre rats. This indicates optogenetic stimulation of PV+ interneurons in the BLA attenuated the IED. In Experiment 2, we used fiber photometry to examine how noradrenergic signaling affects BLA PV+ activity. PV-Cre rats expressing AAV-DIO-jGCaMP8f-GFP in the BLA underwent immediate extinction after systemic propranolol (PROP, 10 mg/kg, i.p.) or vehicle (VEH) injections. Consistent with previous work, PROP attenuated the IED. Interestingly, we found that fear conditioning produced long-term reductions in calcium activity in PV+ neurons and this was attenuated by PROP. Taken together, these findings indicate that stress-induced increases in noradrenergic signaling increases BLA hyperexcitability and impairs extinction learning by inhibiting BLA PV+ interneurons.

Disclosures: S. Plas: None. **K. Crayton:** None. **S. Maren:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

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Program #/Poster #: PSTR084.12/Y12

Topic: H.01. Fear and Aversive Learning and Memory

Support: R01MH065961

Title: Role of NMDA Receptors in the Nucleus Reuniens in the Acquisition and Extinction of Fear Memory

Authors: *A. ARELLANO PEREZ[1], A. P. ABRAMENKO[1], T. TUNA[1,3], S. MAREN[2,1];
[1]Beckman Inst., [2]Psychology, Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: Recent work from our laboratory has demonstrated the critical role of the nucleus reuniens (RE), a midline thalamic hub connecting the hippocampus (HPC) and medial prefrontal

cortex (mPFC), in the acquisition and retrieval of fear memories. Specifically, muscimol-induced inactivation of the RE before conditioning impairs the retrieval of contextual fear memory, resulting in less precise memory recall. This impairment appeared to be state-dependent, as this effect can be rescued when RE is inactivated, suggesting that RE activity during both encoding and retrieval phases is essential for accurate fear memory processing. Building on these findings, we hypothesized that NMDA receptors within the RE are also crucial for the acquisition and suppression of contextual fear. Adult male and female Long-Evans rats were surgically implanted with a single midline guide cannula targeting the RE. Following recovery, animals received intra-RE infusions of either saline (SAL) or the NMDA receptor antagonist APV (2.5µg/µl, 0.3 µl per side; n = 5-8 per group) immediately prior to auditory fear conditioning. The following day, rats underwent a context retention test after either SAL or APV infusions in a factorial design. Consistent with previous work, APV infusions prior to conditioning caused a significant impairment in contextual freezing during the retention test. However, this impairment was eliminated when rats were tested after APV infusions. In other words, deficits in the acquisition of contextual freezing were state-dependent. Interestingly, APV infusions alone during retention testing did not cause a state-dependent generalization decrement. Furthermore, APV infusions did not affect auditory fear memory retrieval. These findings suggest that RE activity is critical for the context-dependence of fear memory. Future research will investigate the neural pathways through which RE contributes to state-dependent encoding of contextual fear memories.

Disclosures: A. Arellano Perez: None. **A.P. Abramenko:** None. **T. Tuna:** None. **S. Maren:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR084.13/Y13

Topic: H.01. Fear and Aversive Learning and Memory

Title: Supramammillary nucleus neuronal activity to aversively conditioned stimulus reflects fear expression

Authors: *T. TUNA[1,3], F. A. G. MOURÃO[1], S. MAREN[2,1];
[1]Beckman Inst., [2]Psychology, Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: The supramammillary nucleus (SUM) of the hypothalamus is connected with many of the brain regions involved in fear and extinction memory processing, including the medial prefrontal cortex, hippocampus, amygdala, and the nucleus reuniens of the thalamus. The SUM, an oscillatory pacemaker in the brain, regulates the frequency of hippocampal theta oscillations which are critical for learning and memory processes. However, the role of the SUM in fear learning and memory processes remains largely unexplored. Here we aimed to characterize

neuronal activity of the SUM during Pavlovian fear conditioning and extinction. To this end, we performed *in vivo* electrophysiological single-unit and local field potential (LFP) recordings from the SUM in adult male and female rats that underwent habituation, auditory fear conditioning, context extinction, cued extinction, and extinction retrieval. Extracellular single-unit recordings revealed that SUM neurons do not respond to neutral tones during habituation but acquire conditioned stimulus (CS)-evoked responses during conditioning. CS-evoked firing was maximal during fear retrieval and decreased over extinction trials and days and remained low during extinction retrieval. Moreover, CS-evoked firing was positively correlated with freezing behavior. We did not record from any SUM neurons that increased responding over the course of extinction. LFP recordings revealed shifts from delta (2-4 Hz) to low theta (4-8 Hz) oscillations during the transition from fearful freezing to non-freezing periods. These shifts occurred during the fear retrieval session, but not habituation or extinction retrieval. Collectively, these results present a characterization of SUM neuronal responses to aversively conditioned stimuli, suggesting that SUM activity is congruent with fear expression. The SUM, a highly overlooked structure in fear research, may prove a potential therapeutic target for the treatment of fear- and trauma and stressor-related disorders.

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Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

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Topic: H.01. Fear and Aversive Learning and Memory

Support: This work was supported by startup funds from the Iowa Neuroscience Institute and the Department of Psychiatry at the University of Iowa.

Title: Cntnap2 has opposing effects on fear learning and extinction learning

Authors: *H. JANOUSCHEK[1], R. J. TAUGHER-HEBL[1,2], A. EAGEN[1], A. TOWNSEND[1], H. C. BERGSTROM[3];
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Abstract: Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that it is not only characterized by deficits in social interaction and restricted repetitive behavior, but that is also associated with increased levels of fear and anxiety, which impair quality of life. The contactin-associated protein-like 2 (*Cntnap2)* gene encodes for the contactin-associated protein-like 2 (CASPR2) protein, a neurexin that has been associated with synapse formation and stabilization, and ASD. CASPR2 is highly expressed in limbic circuitry, including amygdala and hippocampus, regions essential for fear learning, memory storage, and extinction. Here, we

asked what role CASPR2 plays in fear learning and fear memory extinction. Methods: To probe the role CASPR2 plays in fear learning and extinction adult wild-type (n=15) and *Cntnap2* knockout (n=19) mice of both sexes were exposed to Pavlovian auditory cue fear conditioning and fear memory extinction. During acquisition, mice received 3 cue-shock pairings in context A. Starting the day after acquisition, mice underwent 4 days of fear memory extinction training in context B with 50 unreinforced cue presentations each day. 5 days after fear memory acquisition, mice were tested for fear extinction retention with 3 cue presentations, also in context B. We normalized extinction retention by calculating an extinction-retention index from the maximum freezing during CS presentation at the extinction test and the mean freezing during the first 2 CS presentations on the first day of extinction training. Mixed effects linear regression analysis was used to test for genotype differences. The model controlled for potential sibling correlations by including a random effect for litter. We controlled for potential sex effects by including fixed covariate effects for sex and for sex-by-genotype interactions. Results: *Cntnap2* knockouts froze less during fear memory acquisition ($p>0.001$). However, both genotypes froze similarly during the first two cues presented at the first day of extinction training ($p=0.356$), indicating a similar cued response. However, the extinction memory of knockouts was retained more than in wildtypes, resulting in a highly significant difference in extinction-retention ($p>0.001$). Conclusion: Our results suggest that CASPR2 differentially effects fear-learning and extinction learning.

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Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

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Program #/Poster #: PSTR084.15/Y15

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIH Grant R15MH127534-01
NSF Grant 2320195

Title: Can generalized stimuli extinguish a conditioned fear response?

Authors: *A. DAM[1], A. GWYN[2], S. ZWEIG[2], T. K. TSUKUDA[2], T. PASERMAN[2], M. GARCIA[2], A. CONWAY[2], R. GUTIERREZ[2], H. C. BERGSTROM[2];
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Abstract: Exposure therapy is the “gold-standard” cognitive behavioral approach for treating mental illness, including a spectrum of anxiety disorders, stress- and trauma-related disorders, and phobias. Despite this, exposure therapy does not work for all patients, suggesting our currentunderstanding of mechanism is insufficient. Exposure therapy involves repeatedly

“exposing” individuals to stimuli associated with the things they fear. The mechanisms of exposure therapy are based on principles of extinction. While this experimental model has significantly advancedour understanding of extinction mechanisms per se, it does not reflect clinical reality. In the clinic, individuals are typically presented with stimuli that are like or related, but not identical, to the original fear-related conditioned stimulus (i.e., the CS). These stimuli are known asgeneralization stimuli (GS); they have the capability to elicit a conditioned response (CR) even though they differ from the CS. It is possible that a GS can extinguish a CS to some extent, yet the clinical efficacy is variable and brain mechanisms completely unknown. The goal of this project is to identify whether GS can extinguish a CS in a preclinical mouse model. We hypothesize that the degree of extinction using a generalization stimulus depends on the relatedness (i.e., tone frequency or pitch) of GS to the CS. To test this question, adult male andfemale ArcCreER T2 x eYFP mice were fear conditioned with the same tone frequency CS. The next day, mice were presented the CS again to test the CR. Mice were then presented with a different tone frequency GS over 2 days in a standard extinction paradigm. Then, the original CS was presented again to test extinction retention. Context renewal and spontaneous recovery procedures were included to test the efficacy of GS to suppress the CS. We found GS sufficient to extinguish a CS. Ongoing experiments are testing GS at further octaves from the CS, to determine the extent to which a “discriminated stimulus” extinguishes a CS relative to GS.

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Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR084.16/Y16

Topic: I.04. Executive Functions

Support: IITR/SRIC/2741

Title: Inhibitory control training improves fear extinction and extinction recall

Authors: *K. DAR[1], M. K. ASTHANA[2];
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Abstract: Inhibitory learning forms an important component of fear extinction. Prior studies suggest that low inhibitory capacity might delay fear extinction, due to the slower formation of new inhibitory associations (CS+- no UCS) resulting in expression of excitatory association (CS+- UCS). Such excitatory associations prompt automatic fear responses, which are difficult to withhold intentionally and might hinder extinction. Inhibition is an executive function, concerned with deliberate inhibition of dominating and automatic responses. Therefore, improving inhibitory learning has the potential to enhance extinction outcomes. We investigated

the effect of brief inhibitory control training through stop-signal task before extinction on fear extinction and extinction recall in comparison to standard extinction group. 51 participants (F=21, M=30, Mage= 23.07, SD=0.563) completed a 3-day differential fear conditioning experiment. Habituation and acquisition occurred on day 1, followed by extinction on day 2 and extinction recall on day 3. Participants were divided into two group on day 2, inhibitory control and extinction (IC-SE) and standard extinction group (SE). The IC-SE group underwent inhibitory control training before extinction. Our results show that conditioned stimulus (CS+) in IC-SE group showed significant reduction in arousal rating ($t(49) = -2.760$, $p = .008$, $d = -.774$) and UCS-expectancy rating significant ($t(49) = -2.211$, $p = .032$, $d = -.620$) during extinction. Further, skin conductance response (SCR) showed a significant groups difference, with higher values in IC-SE group for both CS- and CS+ [$p = .005$, 95% CI (.025, .132)]. Additionally, we observed reduced arousal ratings ($t(49) = -2.548$, $p = .014$, $d = -.715$) and UCS-expectancy ($t(49) = -2.675$, $p = .011$, $d = -.746$) during extinction recall. Our results imply that training on inhibitory control enhances extinction as indicated by lower UCS-expectancy, further since anticipation to encounter the UCS is low, it results in lower subjective arousal. Inhibitory control training aids the formation of novel inhibitory associations (CS+ not followed by UCS) during extinction. Further, better extinction recall could be attributed to better consolidation of extinction under high arousal state as indicated by increased SCR in IC-SE group.

Disclosures: K. Dar: None. **M.K. Asthana:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.17/Z1

Topic: H.01. Fear and Aversive Learning and Memory

Support: UMOR APSF to NCT

Title: Fear conditioning with partial reinforcement leads to differential prediction and stress responses

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Abstract: Pavlovian fear conditioning has been widely used to understand the neurobiology of fear memories and to model anxiety disorders and post-traumatic stress disorder (PTSD). In Pavlovian fear conditioning, an animal learns to use a cue or context to predict a dangerous or fear-inducing stimulus. However, anxiety and PTSD are characterized by a fear response in situations that do not predict danger. We hypothesize that, in addition to predictive associations, animals learn additional associative components including motivational/affective information. In this study, we first examined whether we could behaviorally identify non-predictive components

of context fear memories. We trained male and female C57Bl6N mice on a continuous- (CRft, 100% reinforcement ratio) or partial- reinforcement (PRft, 50% reinforcement) context fear conditioning schedule, followed by a blocking paradigm with a new tone CS. CRft males, but not PRft males, exhibited blocking to the new CS. Thus, despite strong context fear conditioning, PRft-trained animals did not acquire a strong predictive association. This suggests that some other information must be driving fear responses. Interestingly, CRft trained females showed less robust blocking than males. To further test the hypothesis, we examined stress responsivity in the training context, using ELISA to measure circulating corticosterone (CORT). Here, PRft-trained males showed elevated CORT compared with CRft animals, suggesting a different - more stressful - response to memory retrieval in PRft-trained mice. We next hypothesized that if PRft contributes to less predictive associations, it should result in more anxiety-like behaviors in other contexts. In the open field test, PRft-trained mice exhibited greater anxiety-like behavior than CRft males, again suggesting that decreased prediction results in acquisition of different (not less) memory content. Ongoing work aims to assess differential activity across brain regions and networks in CRft vs PRft. Together, these results demonstrate that context fear conditioning with PRft training results in more "disordered" fear responses, including greater generalization, increased anxiety, and higher stress during fear memory retrieval. This suggests that predictive associations are not sufficient to explain all fear conditioning phenomena, and that identifying what other information is learned during conditioning - including affective/motivational associations - will improve models of fear related disorders including anxiety and PTSD.

Disclosures: J.A. Murray: None. **N.C. Tronson:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.18/Z2

Topic: H.02. Reward and Appetitive Learning and Memory

Title: Contextual and temporal control over novelty-facilitated extinction (NFE)

Authors: *L. J. BRADLEY[1], J. SAINI[2], M. BUKKASAGARAM[3], Z. MEHRZAD[2], A. RAIKAR[2], E. WEBER[2], R. SUMNER[2], V. CAMPESE[4];
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Abstract: Novelty-facilitated extinction (NFE) involves the presentation of a conditioned stimulus (CS) with a novel stimulus (NS). Contrary to the expectations generated by popular models of associative learning, including a novel stimulus during the extinction of conditioned Pavlovian fear has been reported to facilitate response reduction over training as well as in follow up tests. These results are encouraging for researchers seeking effective behavioral treatments to attenuate maladaptive fear and anxiety. However, these reports often lack rigorous testing to determine the efficacy of this treatment against the many documented extinction

recovery effects (e.g., renewal, spontaneous recovery, reinstatement). To study whether ABA renewal is attenuated by NFE, two distinct contexts were created to establish a generalization of the processes and eliminate environmental bias. Thirty-one food deprived rats (16 males, 15 females) underwent two days of magazine training, one day in each context. To establish lever press responding, four days of continuous reinforcement were followed by variable ratio training (VR10, VR20, VR30). Subjects then underwent Pavlovian fear conditioning where tone and noise cues were each paired with shock in separate contexts. After which, in extinction, subjects were then separated into 2 groups- 1 group which involved NFE treatment including a light stimulus (8 males, 7 females) and a group with only tone and noise alone extinction. Both of these treatments were employed during Pavlovian Instrumental Training (PIT) testing. During transfer tests of the first session (i.e. PIT), the male rats in ABB maintained a similar suppression ratio in both the NFE and non-NFE rats, (NFE = 0.563, EXT = 0.431) respectively. In the ABA tests, the results were comparable (NFE = 0.544, EXT = 0.478). ABA responding in NFE females (M = 0.73) was higher than standard EXT females (M = 0.55), while ABB responding was more comparable (M = 0.539 and M = 0.638 respectively). These findings suggested that the extinction type (i.e., NFE vs. standard) had minimal impact on the renewal of the fear. More work is needed to fine tune the conditioned suppression procedure, but early work suggests little advantage of NFE treatment in reducing context dependent recovery. Ongoing studies will replicate and extend this analysis into magazine approach and other renewal/recovery paradigms.

Disclosures: L.J. Bradley: None. **J. Saini:** None. **M. Bukkasagaram:** None. **Z. Mehrzad:** None. **A. Raikar:** None. **E. Weber:** None. **R. Sumner:** None. **V. Campese:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.19/Z3

Topic: H.01. Fear and Aversive Learning and Memory

Support: MD007579 (B.R.A.I.N. CORE)
GM082406 (NIH RISE PROGRAM)
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Puerto Rico Science, Technology and Research Trust Catalyzer Research Grant
Fundacion Intellectus

Title: Targeting Neurogranin and Microtubule-Associated Protein Tau in the Infralimbic Cortex to Improve Fear Extinction in Single Prolonged Stress-Exposed Female Rats

Authors: *N. IRIZARRY-MÉNDEZ[1], A. HERNÁNDEZ[2], M. CRUZ[3], M. COLON[3], J. T. PORTER[4];

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Abstract: Traumatic experiences can induce long-lasting changes in the brain, impairing fear extinction and increasing vulnerability to trauma-related disorders. Although these disorders are more prevalent in females, the molecular mechanisms underlying impaired fear extinction in this population remain poorly understood. In our previous work, we found that adult female rats exposed to single prolonged stress (SPS) and classified as stress-susceptible, based on extinction performance showed a distinct infralimbic cortex (IL) proteomic signature compared to resilient rats. Among the differentially expressed proteins, neurogranin and microtubule-associated protein tau (MAPT), two proteins associated with learning and memory were elevated in susceptible rats. These findings led us to hypothesize that elevated neurogranin or MAPT may contribute to extinction impairments. To assess the potential contribution of these proteins to extinction impairments, we infused an AAV9 vector expressing 3 different shRNAs targeting either neurogranin or MAPT, along with a fluorescent reporter (mCherry) into the IL of adult female rats. One month later, female rats were exposed to SPS, followed by auditory fear conditioning and extinction training. Brain tissue was collected for immunofluorescence analysis to confirm infusion site and knockdown efficiency. Preliminary results show that reducing the expression of neurogranin or MAPT in the IL improves extinction recall in SPS-exposed females, suggesting a role for these proteins in mediating stress-induced extinction deficits. These findings highlight neurogranin and MAPT as potential molecular targets for enhancing extinction in females following traumatic stress.

Disclosures: N. Irizarry-Méndez: None. **A. Hernández:** None. **M. Cruz:** None. **M. Colon:** None. **J.T. Porter:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.20/Web Only

Topic: H.01. Fear and Aversive Learning and Memory

Support: PAPIIT-UNAM Grant IN221025

Title: The histone deacetylase inhibitor sodium butyrate and corticosterone enhance extinction of contextual fear conditioning

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Abstract: Extinction is a form of cognitive and behavioral flexibility that allows animals to adapt their behavior to new circumstances. Evidence shows that glucocorticoids, stress hormones, can modulate the acquisition and extinction of contextual fear conditioning. In particular, glucocorticoids, such as corticosterone (CORT) facilitate extinction, but the associated cellular mechanisms remain poorly explored. Chromatin modifications, including post-translational histone modifications, represent a key molecular pathway in the regulation of gene expression required for long-term synaptic plasticity and memory formation. Studies demonstrate that systemic administration of a histone deacetylase inhibitor, such as sodium butyrate (NaB), facilitates gene transcription and enhances memory consolidation. However, it remains unclear whether histone deacetylases are a mechanism involved in the glucocorticoid-mediated facilitation of extinction. To evaluate this, we trained rats in a contextual fear conditioning task and 48 hrs after subjected them to an extinction session. Immediately after CORT (10 or 30 ng), NaB (0.3 or 1 µg), or vehicle was administered into the infralimbic cortex. Extinction retention was assessed 48 hrs later. In a separate group, the glucocorticoid antagonist RU486 (10 ng) was administered 5 minutes before extinction, followed immediately by NaB (1 µg). Our results showed that both CORT 10 ng and 30 ng groups exhibited more efficient extinction compared to control. The NaB 1 µg group also showed enhanced extinction relative to the NaB 0.3 µg and vehicle groups. Additionally, the vehicle-NaB 1 µg group showed more efficient extinction then the vehicle-vehicle, RU486-vehicle, and RU486-NaB 1 µg groups. These findings suggest that glucocorticoids facilitate memory extinction, in part, by increasing histone acetylation and promoting gene expression via the glucocorticoid receptor activation in the infralimbic cortex.

Disclosures: A. Fuentes Ibañez: None. **M.M. Monroy:** None. **O.A. Gutierrez:** None. **G.L. Quirarte:** None. **G.R. Roldan:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.21/Z4

Topic: H.01. Fear and Aversive Learning and Memory

Support: University of Massachusetts Boston Retired Faculty Council Endowed Faculty Career Award

Title: Influence of Lactobacillus rhamnosus on anxiety and fear mechanisms in male and female rats.

Authors: *C. E. HARTSGROVE[1,2], G.-M. WALKER[2], K. D. SILVA[2], K. NUNEZ[3], K. ALEJOS[2], M. JOSEPH[2], K. HARRIS[5], J. LIN[4], K. CADET[2], R. MANIANI[2], A. TRAN[2], L. Y.

MAENG[6];
[1]Univ. of Massachusetts, Boston Dept. of Psychology, Abington, MA; [2]Psychology, [4]Biochem., [3]Univ. of Massachusetts Boston, Boston, MA; [5]Psychology, Univ. of Massachusetts, Boston, Boston, MA; [6]Psychology, Univ. of Massachusetts, Boston, MA

Abstract: The gut-brain axis has been implicated as a critical mediator of several psychiatric disorders, including anxiety and post-traumatic stress disorder (PTSD). Anxiety and PTSD are characterized by impaired fear extinction, the learned inhibition of fear after a threat is no longer present. In animal models of PTSD, antibiotic-induced disruption of the gut microbiome impairs fear extinction differently in males and females. In contrast, administering probiotics - bacteria that benefit the health of the host - has recently emerged as an innovative potential intervention for anxiety. In particular, the probiotic Lactobacillus rhamnosus (LR) has been shown to significantly reduce anxiety-like behavior and hormonal stress responses, but the effect of this probiotic on fear extinction is still unclear. Despite evidence from human and animal research on sex differences in fear extinction memory, LR effects on fear have only been studied in juvenile male rats. This leaves questions about whether the anxiolytic effects of LR are sex- and/or age-dependent. The current study addressed this gap by investigating the effects of LR on anxiety-like behaviors, fear extinction memory, and hormonal stress responses in adult male and female rats. Forty-eight Sprague Dawley rats received daily oral administration of LR or vehicle (PBS) for 21 days. Anxiety-like behavior was examined before and after treatment using the elevated plus maze (EPM). Serum corticosterone levels (CORT) were quantified using ELISA before and after the EPM. A 3-day auditory cued fear conditioning and extinction paradigm was used to examine fear extinction memory. While no significant effects of sex or probiotic treatment were observed in fear behavior, a 2x2 ANOVA revealed that females had a significantly greater open/closed arm entries ratio [$F(1, 44)=6.659$, $p=0.008$] and duration ratio [$F(1, 44)=7.602$, $p=0.008$] in the EPM, indicating that female rats exhibited less anxiety-like behavior compared to males. LR administration also significantly decreased time spent in the closed arms of the EPM in both sexes [$F(1,44)=4.425$, $p=0.041$]. In addition, females exhibited a significantly lower percent change in serum CORT levels after EPM compared to males [$F(3, 36)=11.835$, $p=0.001$]. Post hoc pairwise comparisons further revealed that LR-treated females had a significantly lower CORT response than LR-treated males [$p=0.002$]. Together, these findings suggest that there are sex differences in the effects of LR on anxiety-like behavior and CORT response to acute stress in adult male and female Sprague Dawley rats. Ongoing analyses will examine LR effects on neural mechanisms involved in fear extinction memory.

Disclosures: C.E. Hartsgrove: None. **G. Walker:** None. **K.D. Silva:** None. **K. Nunez:** None. **K. Alejos:** None. **M. Joseph:** None. **K. Harris:** None. **J. Lin:** None. **K. Cadet:** None. **R. Maniani:** None. **A. Tran:** None. **L.Y. Maeng:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.22/Z5

Topic: H.01. Fear and Aversive Learning and Memory

Title: Hippocampal theta dynamics during threat processing in trace contextual fear conditioning

Authors: *K. D. STEVANOVIC[1], E. E. THOMSON[2], J. D. CUSHMAN[3];
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[3]Neurobio. Lab., Natl. Inst. of Envrn. Hlth. Sci., Durham, NC

Abstract: The hippocampal theta rhythm has long been studied for its roles in memory and spatial navigation, yet the function of its lower-frequency component, Type II theta (4-7 Hz) remains poorly understood. Type II theta, typically observed during immobility and sensitive to cholinergic antagonism, has been implicated in anxiety and fear, but its precise contribution and underlying mechanisms are unclear. The objective of this study was to elucidate the role of lower-frequency theta oscillations in hippocampus-dependent fear learning, with a focus on threat processing. We conducted wireless local field potential (LFP) recordings from the stratum lacunosum-moleculare of dorsal CA1 in wild-type C57BL/6J mice during a trace fear conditioning paradigm, which requires hippocampal processing. Mice received five tone (20 s, 75 dB 2800 Hz)-shock (2s, .5 mA) pairings with a 20s trace interval separating the tone and shock. Freezing behavior, a measure of learned fear and threat assessment, was quantified throughout acquisition, context, and tone tests. LFP spectrograms were analyzed to extract peak theta frequencies, and kernel density estimation was used to objectively distinguish low (Type II, 4-7 Hz) and high (Type I, 7-10 Hz) theta modes. A theta ratio, reflecting the relative dominance of low vs. high theta, was computed moment-by-moment for each animal and correlated with behavioral (freezing, motion, acceleration) and task epoch parameters. Results showed that, during successful acquisition and retrieval of fear memory, there was a significant increase in Type II theta power, especially during freezing behavior, consistent with a role for this oscillation in threat processing. In contrast, high-frequency theta was more prominent during active movement, as expected. The theta ratio robustly tracked behavioral state transitions, with sharp increases in low theta corresponding to periods of freezing and threat anticipation. Our data lets us investigate whether Type II theta represents a distinct oscillatory state or simply the loss of Type I due to immobility. In conclusion, our findings demonstrate that lower-frequency (Type II) theta oscillations in dorsal CA1 are strongly linked to the distinct processing of threat and fear assessment, as evidenced by their dominance during freezing in trace fear conditioning. This work highlights the unique role of Type II theta in orchestrating hippocampal network dynamics during threat processing and provides new analytic tools for dissecting theta substructure in real time during complex behaviors.

Disclosures: K.D. Stevanovic: None. **E.E. Thomson:** None. **J.D. Cushman:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: PSTR084.23/Z6

Topic: H.01. Fear and Aversive Learning and Memory

Support: NIH Grant R21 MH121772

Title: Chemogenetic inhibition of the claustrum during contextual and cued fear recall

Authors: *K. W. HE[1], R. G. PARSONS[2];
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Abstract: The claustrum (CLA) is a thin, elongated subcortical structure that shares bidirectional connections with many regions of the neocortex and selected subcortical regions, including the amygdala and hippocampus. This extensive connectivity positions the claustrum as a key participant in a variety of fundamental brain functions and prior work has implicated it in consciousness, sleep, memory, attention, and salience processing. Prior work from our lab showed that the claustrum was activated by the recall of both cued and contextual fear in rats. However, studies are needed to test if manipulation of neural activity in claustrum affects the expression of cued and contextual fear conditioning. Here, we utilized chemogenetic manipulation to better understand the role of the claustrum in. In a preliminary experiment, rats underwent surgery to receive 0.2ul of AAV8-CamKIIa-hm4Di-mCherry or AAV8-CamKIIa-eGFP injection bilaterally into claustrum 5 weeks prior to the behavioral experiment. On training day, rats were conditioned in Context A with 2 tone-foot shock pairings. During initial Context A testing, rats received an intraperitoneal injection of deschloroclozapine (DCZ; 0.1mg/kg) 30 minutes prior to Context A exposure. During second context A testing, rats were exposed to Context A again for 5 minutes without DCZ injection. Our results showed that there was no significant difference in freezing between rats that received hm4Di and rats that received eGFP during initial and second Context A exposure. One week following Context A testing 2, rats were exposed to Context B with 4 presentations of tone for cued fear recall. Rats in both groups received a DCZ injection 30 minutes prior to auditory fear recall during initial tone testing and without a DCZ injection on second testing at 48 hours. Our results showed that there was no significant difference in freezing between rats that received hm4Di and rats that received eGFP during initial and second tone testing. However, we observed that during initial testing, rats that received hm4Di exhibited increased freezing during the expression of cued fear. Experiments are ongoing to determine if the increased freezing that we observed during the expression of cued fear after blocking neural activity in the claustrum reflects increased expression of fear or an enhancement of early extinction learning.

Disclosures: K.W. He: None. **R.G. Parsons:** None.

Poster

PSTR084: Fear and Extinction Learning: Neural Mechanisms and Behavior

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR084.24/Z7

Topic: H.01. Fear and Aversive Learning and Memory

Support: FAPESP 2023/02122-8
FAPESP 2022/02986-0
CAPES-PROEX 001
CAPES 88887.696758/2022-00
CNPq 305541/2022-6

Title: Role of corticosterone and ventral tegmental area corticosteroid receptors in contextual fear conditioning

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Abstract: The hypothalamic-pituitary-adrenocortical (HPA) axis, which promotes corticosteroid release, can be activated by conditioned aversive stimuli. Conditioned freezing has been associated with increased dopamine release in the ventral tegmental area-basolateral amygdala (VTA-BLA) pathway. Previously, we showed systemic metyrapone (a corticosterone synthesis inhibitor) prevented dopamine release enhancement in the VTA-BLA and reduced freezing. A follow-up study revealed intra-VTA spironolactone (a mineralocorticoid receptor [MR] antagonist), but not mifepristone (a glucocorticoid receptor [GR] antagonist), similarly suppressed dopamine release and attenuated freezing. Both studies used cued fear conditioning in male rats. The present study investigates the role of corticosterone signaling via MR and GR receptors in the VTA in the expression of contextual fear conditioning in male and female rats. The experiments involved 272 adult Wistar rats (105 males, 167 females; CEUA protocols 9367190422 and 2425060423). Experiment 1 involved intraperitoneal metyrapone (0, 30, or 60 mg/kg). Experiments 2 and 3 assessed intra-VTA spironolactone or mifepristone (0, 5, or 10 ng/0.2 μL). All experiments used a 3-day contextual fear protocol: Day 1, training with footshock presentations; Day 2, treatment before test session to assess freezing behavior; Day 3 drug-free retest. Data were analyzed using one-way ANOVAs followed by Tukey's post hoc tests, with significance set at $p < 0.05$. A significant reduction in freezing was observed in male rats treated with the highest dose of systemic metyrapone (60 mg/kg) during the test session ($F_{2,40} = 3.30$, $p < 0.05$). No such effect was found following intra-VTA administration of MR ($F_{2,38} = 1.35$, $p > 0.05$) or GR ($F_{2,18} = 0.39$, $p > 0.05$) antagonists. In females, no significant effects were detected in any condition (metyrapone: $F_{2,88} = 1.84$, $p > 0.05$; spironolactone: $F_{2,49} = 1.27$, $p > 0.05$; mifepristone: $F_{2,21} = 1.47$, $p > 0.05$). These results suggest that corticosterone plays a sex-dependent role in the expression of contextual fear, with systemic blockade reducing freezing only in males. The lack of effect following intra-VTA antagonism suggests involvement of other brain regions in mediating this behavior.

Disclosures: L.M. Taguchi: None. **J.N. Chiossi:** None. **A.R. de Oliveira:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.01/Z8

Topic: H.08. Drugs of Abuse and Addiction

Support: Japanese Grant-in-Aid for Scientific Research :KAKENHI 20k17793

Title: Relationship between biased agonism and opioid tolerance or adverse events

Authors: *S. KOKUBU[1], H. HOSHIJIMA[2], N. OHASHI[3], S. YAMAGUCHI[1], T. L. YAKSH[4]; [1]Dokkyo medical Univ., Tochigi, Japan; [2]Tohoku Univ. Grad. Sch. of Dent., Miyagi, Japan; [3]Anesthesiol., Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata, Japan; [4]UCSD Anesthesia Lab. 0818, La Jolla, CA

Abstract: Opioid tolerance and various other side effects are adverse events that impede pain treatment, and their resolution is an urgent issue. From the viewpoint of biased agonism which has been proposed in recent years, for elucidate the mechanism of opioid tolerance at the receptor level, complement the classical RAVE hypothesis, and establish an effective indicator of OS, we investigated tolerance, cross-tolerance, and recovery from tolerance (thermal escape latency) through opioid switching between four drugs: MOR G protein biased agonist PZM21, minimally internalizing morphine, highly internalizing fentanyl, and DAMGO, which is commonly used as a control and induces sufficient G protein signaling and β-arrestin signaling, using SD rats with intrathecal catheter. In addition, we also observed physical dependence (withdrawal symptom score) and muscle rigidity (straub tail), and verified the effects of biased agonism. As a result, the group switching from PZM21 to morphine showed no recovery of tolerance, whereas the groups switching to fentanyl or DAMGO showed significant recovery of tolerance. On the other hand, the DAMGO pre-treatment group showed no significant tolerance, but significant tolerance was observed across all groups after switching to other drugs. Muscle rigidity exhibited reversible effects across all groups but disappeared to show after the onset of analgesic tolerance, with a tendency to reappear as analgesic tolerance recovered. Withdrawal symptoms associated with naltrexone administration reached their maximum on the last day for all drugs, indicating physical dependence, regardless of recovery of tolerance after switching. From the above, even if bias agonists have an effective analgesic property, suggest a propensity to induce analgesic tolerance and cross-tolerance, and analgesic tolerance induced by GP biased agonists or β-arrestin biased agonists may recover upon switching to each other. Physical dependence depends on the duration of administration, suggesting that the little indication of influence of bias characteristics of the agonist. On the other hand, Muscle rigidity occurs concurrently with analgesic effects and may decrease alongside analgesic tolerance, implying a potential dependency on G protein signaling transmission. In opioid switching conducted alongside analgesic tolerance, biased factor could serve as useful indicators, and the development of balanced agonists or combinations of β-arrestin bias agonists and G protein bias agonists may be useful from the perspective of analgesic tolerance.

Disclosures: S. Kokubu: None. **H. Hoshijima:** None. **N. Ohashi:** None. **S. Yamaguchi:** None. **T.L. Yaksh:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.02/Z9

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant UG3DA050271

Title: Evaluation of two novel compounds compared to methadone on morphine withdrawal in a rat model of physical dependence

Authors: *M. BURNETT[1], E. BILLINGHAM[1], N. BAXTER[1], M. PEARCE[1], D. RIAL[1], S. P. VICKERS[1], N. MIRZA[1], L. KIRKPATRICK[2], L. PESTANO[2];
[1]Sygnature Discovery, Nottingham, United Kingdom; [2]Ensysce Biosci., La Jolla, CA

Abstract: Opioid addiction is a significant public health concern, and its pharmacological therapeutic management requires better options. Methadone is frequently used in opioid substitution therapies, but adverse events related to its use remain a public health concern. Two trypsin activated (TAAP™) methadone prodrugs (Levo-1-ME and Levo-2S-ME) are in development for opioid substitution therapy. First, we determined the tolerated dose of Levo-1-ME and Levo-2S-ME in morphine dependent rats and then evaluated the efficacy of both drugs, compared to methadone, in morphine withdrawal. Following baseline, male Sprague Dawley rats were administered oral morphine (30 mg/kg) twice daily for 14 days to induce morphine dependence. Abrupt cessation of morphine administration initiated withdrawal. Body weight, food and water intake and 44 behaviours were assessed daily. Morphine withdrawal was characterised by hyperphagia, hyperdipsia, hunched posture, arched back, irritability, piloerection, increased respiration and diarrhoea. In the tolerability study, an escalating dose of Levo-1-ME (60 - 100 mg/kg) and Levo-2S-ME (60 - 80 mg/kg) were administered orally once daily during morphine withdrawal, to assess tolerability (n=2-3). Dose was increased if withdrawal parameters mirrored vehicle or decreased if adverse effects were present. Body weight and food and water intake resembled methadone treatment at Levo-1-ME (100 mg/kg) and Levo-2S-ME (80 mg/kg) without adverse effects. The efficacy study compared methadone (15 mg/kg, twice daily, oral) to Levo-1-ME and Levo-2S-ME (tolerable doses: 80 mg/kg, once daily, or 60 mg/kg, twice daily, oral) during morphine withdrawal. Methadone reduced hyperphagia, hyperdipsia, hunched posture, arched back, irritability, piloerection, increased respiration and diarrhoea. Additional behaviours seen with methadone treatment that were not present during morphine withdrawal were ataxia, Straub tail, increased locomotion, increased reaction to sound, explosive movements, exophthalmos, stereotypies, salivation, consumption of bedding and decreased respiration. All doses of Levo-1-ME and Levo-2S-ME were found to reduced hyperphagia, hunched posture, arched back, irritability, increased respiration and diarrhoea. The prodrugs differed from methadone in that they did not induce morphine or methadone related behaviours during withdrawal, and in particular none of the potentially harmful observations seen with methadone described above. The data suggests that Levo-1-ME and Levo-2S-ME may be useful new tools for opioid substitution therapy with a decreased side effect profile versus methadone.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.03/Z10

Topic: H.08. Drugs of Abuse and Addiction

Support: U01DA050243

Title: Enhanced naloxone-induced conditioned place aversion following high-dose oxycodone in Zhx2 knockout mice

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Abstract: The opioid epidemic remains a major public health concern as opioid-use and overdose-related deaths continue to rise, with the prescription opioid, oxycodone **(OXY)**, being a major historical and ongoing part of this crisis. A major driver of continued opioid use in patients with opioid use disorder (OUD) is to prevent withdrawal during abstinence. OUD is a heritable substance use disorder with genetic factors that can influence both acute opioid behavioral sensitivity and withdrawal. We previously mapped and validated zinc-fingers and homeobox 2 **(Zhx2)** as a quantitative trait gene underlying increased brain oxymorphone **(OMOR)**, in BALB/cJ females (Beierle et al., 2022; *JPET;* Lynch et al., 2025; *JPET*). OMOR is a much more potent and efficacious metabolite and Zhx2 loss-of-function induced an increase in brain OMOR levels in female mice. Here, we tested the hypothesis that Zhx2 knockout would increase OXY-induced withdrawal in a conditioned place aversion **(CPA)** paradigm. Mice were assigned to OXY/naloxone **(NLX)** or saline/saline **(SAL)** control groups. On Day**(D)**1, all mice received SAL at 0800h in the home cage and again at 1200h and were assessed for initial preference/aversion for 30 min in a two-sided apparatus. On D2 and D4, mice received OXY (40 mg/kg; IP) at 0800h and NLX (1 mg/kg; IP) at 1200h and were confined to the right side for 30 min. Controls received SAL at both time points. On D3 and D5, mice received SAL at both timepoints and were confined to the left side for 30 min. On D8, all mice received SAL at both timepoints and were assessed for drug-free NLX-CPA for 30 min. On D9, OXY/NLX mice received SAL at 0800h and NLX as a reminder injection at 1200h, while controls received SAL at both timepoints and once again, mice were tested for NLX-CPA. As predicted, Zhx2 knockout mice showed enhanced NLX-CPA as indicated by significantly less time spent on the NLX-paired side. We are currently assessing brain OMOR in Zhx2 knockout mice following high-dose

OXY and future studies will assess changes in brain reward threshold during spontaneous OXY withdrawal, as well as computer vision analysis of behavioral differences in the presence and severity of withdrawal phenotypes across KO genotypes. Overall, our results support Zhx2 loss-of-function in enhancing brain OMOR levels and multiple OXY addiction model behaviors.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.04/Z11

Topic: H.08. Drugs of Abuse and Addiction

Title: Assessment of fentanyl-xylazine combination on respiratory function in rats

Authors: *T. TORGBENU[1], J. ZUARTH GONZALEZ[1], L. R. MCMAHON[2], S. OBENG[1]; [1]Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX; [2]Pharmaceut. Sci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Over the past decade, fatal opioid overdoses in the United States have nearly tripled, with synthetic opioids - notably fentanyl - implicated in about 92% of these deaths. Fentanyl acts primarily via activation of the mu-opioid receptor, producing both analgesia and profound respiratory depression. Alarmingly, the lethality of illicit fentanyl has been exacerbated by the emergence of xylazine, an alpha2-adrenegic receptor agonist commonly used as a veterinary sedative, now increasingly detected as an adulterant in street drugs. This study investigated the effects of fentanyl, xylazine and their combination in the ratio of 1:3 (ratio of the absolute amounts of fentanyl to xylazine used by people who inject drugs) on respiratory function using whole-body plethysmography in jugular catheter-implanted male and female Sprague Dawley rats. The drugs were administered intravenously, and respiratory parameters (frequency, tidal volume, and minute volume) were monitored to assess the extent of respiratory depression. A within-subject, repeated-measures experimental design was employed, with treatment counterbalanced and a one-week washout period between sessions. The study consisted of two experimental phases: in the first phase, the same group of subjects received fentanyl (0.01, 0.032, and 0.056 mg/kg) and Xylazine (0.1 and 0.178 mg/kg) and combination of Fentanyl (0.056 mg/kg) + Xylazine (0.178mg/kg). To evaluate potential reversal of respiratory depression, naloxone (mu opioid receptor antagonist) and atipamezole (alpha2-adrenergic receptor antagonist) were administered 10 min post-treatment at a dose of 1.0 mg/kg in different sessions during the second phase. Xylazine alone did not result in significant respiratory depression. Fentanyl alone reduced minute volume to 29% of baseline within 5 minutes, with recovery to 96% of baseline in 20minutes. However, the combination with xylazine reduced minute volume

to 42% of baseline within 5 minutes, with recovery to only 68% of baseline. Naloxone induced a rapid reversal of respiratory depression induced by fentanyl in combination with xylazine whereas atipamezole resulted in a more gradual recovery. These results suggest that, xylazine alone does not significantly suppress respiration however, its combination with fentanyl exacerbates and prolongs respiratory compromise, complicating clinical intervention in overdose scenarios.

Disclosures: T. Torgbenu: None. **J. Zuarth Gonzalez:** None. **L.R. McMahon:** None. **S. Obeng:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR085.05/Z12

Topic: H.08. Drugs of Abuse and Addiction

Support: RSCA Grant (CSU Chico)

Title: Understanding the impact of polydrug exposure to benzodiazepines and opioids in adolescent mice

Authors: I. OLIVAREZ[1], D. PAPASERGIA[1], *S. BATES[2];
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Abstract: Benzodiazepines and opioids are among the most frequently prescribed and abused drugs in adolescents. However, little is known about their interactions during concomitant use in this age group. Adolescence is a critical period of brain development during which exposure to psychoactive substances such as opioids and benzodiazepines can lead to long-lasting behavioral and neurobiological changes. The current study examined the effects of diazepam (DZP), a benzodiazepine, on morphine-induced behaviors. Adolescent mice were administered chronic morphine to induce dependence and were subsequently tested following diazepam or saline treatment. We examined opioid-induced antinociception, withdrawal symptoms (both somatic and anxiety-like), opioid reward, and social interaction. Additionally, we quantified the gene expression of GABA (gamma-aminobutyric acid), ERK (extracellular signal-regulated kinase), PKC (protein kinase C), and CCK (cholecystokinin) in the striatum after saline or DZP treatment. This study yielded interesting, sex-dependent results. DZP reversed opioid-induced antinociceptive tolerance in male mice only. Morphine-induced withdrawal symptoms were evident by increased jumping and rearing behaviors, but DZP did not attenuate these symptoms in either sex. Furthermore, acute benzodiazepine exposure resulted in increased morphine reward in male mice, as measured by conditioned place preference. Lastly, DZP decreased social interaction in female mice, suggesting an increase in social anxiety. On a molecular level, DZP increased expression of ERK, PKC, and GABA, along with decreased CCK expression, which

points to a compensatory neurochemical response that stabilized neural excitability. These findings suggest that while both sexes undergo comparable neurochemical adaptations, the behavioral ramifications induced by polydrug exposure are sex dependent. These data highlight the importance of targeted prevention strategies for adolescent populations. Furthermore, they demonstrate the dangers of adolescent opioid and benzodiazepine polydrug use and can be of use to clinicians prescribing these drugs as well as practitioners that treat adolescents with substance use disorders.

Disclosures: I. Olivarez: None. **D. Papasergia:** None. **S. Bates:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR085.06/AA1

Topic: H.08. Drugs of Abuse and Addiction

Support: R01 DA045499
T32 DA7237-35

Title: A-438079, a selective P2X7 antagonist, reduces morphine-derived physical dependence, exhibits anxiolytic properties, decreases depressive-like behaviors during morphine withdrawal, and lowers oxycodone-induced hyperlocomotion

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Abstract: Prescription opioids, such as morphine and oxycodone, remain the gold standard for the management of severe pain. Chronic administration of opioids, however, results in numerous deleterious side effects, such as respiratory depression, tolerance, hyperalgesia, physical dependence, and addiction. Additionally, acute withdrawal from opioids causes intense physical discomfort and extended withdrawal leads to anxiety and depressive-like behaviors. Preclinical evidence demonstrates that proinflammatory cytokines contributes to these adverse effects of opioids. The P2X7 receptor is a key regulator of proinflammatory cytokine release as well as neurotransmitters such as glutamate and dopamine. While chronic morphine induces activation of P2X7 and downstream cytokine release, it is unknown if P2X7 activation contributes to these adverse side effects of opioids. In this study, we tested the effects the selective P2X7 antagonist A-438079 against morphine-induced respiratory depression, tolerance, hyperalgesia, physical dependence, anxiety and depressive-like behaviors during withdrawal, respiratory depression, as well as oxycodone-induced hyperlocomotion. Our results indicate that 10 mg/kg, but not 30 mg/kg, significantly reduced acute withdrawal symptoms induced by naloxone. Furthermore, 10 mg/kg A-438079 exerted anxiolytic effects as measured by increases in both open arm time and open arm entries on the elevated plus maze in both morphine-dependent and morphine-naïve

rats. Rats with a history of morphine and 10 mg/kg A-A438079 exhibited significantly lower immobility time on the forced swim test, indicating a reduction in depressive-like behaviors. Treatment with 10 or 30 mg/kg A-438079 did not exacerbate morphine tolerance, hyperalgesia, or respiratory depression in rats. Finally, both 10 and 30 mg/kg A-438079 significantly reduced oxycodone-induced hyperlocomotion in mice, suggesting that P2X7 antagonism may reduce the rewarding properties of opioids. Taken together, these results demonstrate that P2X7 antagonism may provide a therapeutic route against physical dependence, anxiety and depressive-like behaviors, and rewarding properties of opioids.

Disclosures: S.D. Dunn: None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.07/AA2

Topic: H.08. Drugs of Abuse and Addiction

Support: T32-DA017444
R37-DA033397

Title: The mu opioid receptor positive allosteric modulator BMS-986122 attenuates fentanyl withdrawal

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Abstract: Opioid withdrawal syndrome (OWS) is a major contributor to continuing drug use in opioid use disorder (OUD), and clinical management of OWS has proven challenging (Pergolizzi et al., 2019). Additionally, chronic opioid use can result in opioid tolerance and the need to escalate doses, exacerbating opioid dependence and OWS. We have previously described a positive allosteric modulator of the mu-opioid receptor (MOR-PAM), BMS-986122 (BMS122). BMS122 acts at a distinct site on MOR to enhance the potency and or efficacy of orthosteric agonists. We have further shown these compounds increase the activity of endogenously released opioid peptides at MOR. In contrast these compounds administered alone do not cause respiratory depression, constipation, or reward (Kandasamy et al., 2021).
We hypothesized that BMS122 would enhance the activity of MOR agonists and relieve opioid-induced somatic and behavioral symptoms resulting from chronic opioid use including tolerance, and withdrawal. Using an in-vitro functional assay of G-protein coupled receptor activation (GTP γ^{35}S) at human opioid receptors expressed in CHO cells we show that BMS122 (10 μ M) enhances the activity of the enkephalin derivative MOR peptide DAMGO (48.6 nM ± 6.4 DAMGO vs 9.5 nM ± 0.7 DAMGO+BMS122) but does not significantly shift the potency of the endogenous kappa-opioid (KOR) ligand dynorphin (0.68 nM ± 0.3 dynorphin vs 0.44 nM ± 0.1

dynorphin + BMS122). These data suggest BMS122 would enhance the activity of endogenous opioid peptides at MOR to relieve withdrawal symptoms without exacerbating dynorphin-mediated withdrawal-behaviors. Male and female C57Bl/6j mice were made dependent on fentanyl utilizing an osmotic mini pump system to continuously supply 0.3 mg/kg/day fentanyl, After 7 days, 3.2 mg/kg naloxone (NLX) or 3.2 mg/kg NLX in the presence of BMS122 was used to precipitate withdrawal. Somatic signs of withdrawal (jumping, paw tremor, teeth chatter) were measured. Relative to the NLX alone group, NLX + BMS122 significantly reduced jumping, paw tremor and teeth chatter behavior associated with opioid withdrawal (16 ± 3 morphine vs 4 ± 2 BMS122 jumps, 26 ± 4 vs 4 ± 3 paw tremors, and 17 ± 2 vs 4 ± 2 teeth chatters).
Taken together these data suggest positive allosteric modulation of MOR may provide a promising therapeutic strategy to ameliorate withdrawal-induced physical and mood-altering symptoms which are significant clinical challenges to managing OWS. This work was generously funded by T32 DA017444 and R37 DA033397.

Disclosures: D. Jessup: None. **J.R. Traynor:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.08/AA3

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA Grant 047413

Title: The neurobehavioral effects of contingent and non-contingent oxycodone exposure on perineuronal nets in wistar rats

Authors: *F. J. BRESSLER, S. JEWANEE, M. DEJEUX, C. WERNETTE, J. D. NGUYEN; Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: The nonmedical use of oxycodone, a potent and commonly prescribed opioid analgesic, can cause addiction and dependence. To understand the development of prescription opioid use disorder, this study aims to examine how different patterns of oxycodone exposure, whether contingent or non-contingent, affect the brain's structural and functional integrity through the dynamic regulation of perineuronal nets (PNNs). We hypothesized that abstinence following tolerance to repeated injections of oxycodone will change the expression of perineuronal nets (PNNs) in various brain regions including the orbital frontal cortex and somatosensory cortex of male and female rats. Adult male and female Wistar rats were subjected to repeated (twice daily for 7 days) injections of oxycodone (2 or 4 mg/kg, s.c.) or 0.9% saline vehicle. After 14 injections, rats were either subjected to acute (24 hours) or protracted (4 weeks) withdrawal. Thermal antinociception (i.e. tail withdrawal test) was used to validate drug-induced

tolerance. Locomotor activity and withdrawal scoring were also gathered to assess withdrawal-like symptoms. Immunohistochemistry was used to identify Wisteria Floribunda Agglutinin (WFA)-positive perineuronal net structures and Paravalbumin positive interneurons (PV+). The second project investigated the neurobehavioral effects of intravenous self-administration of oxycodone in male and female rats. Adult male and female Wistar rats were individually housed in operant chambers that had two response levers. Access to intravenous self-administration of oxycodone (0.15 mg/kg/inf) or 0.9% saline vehicle was given in short access for 5 sessions/week at a 0.1 mL per infusion volume. Fixed ratio schedule was set in place to train the rats to press one lever for drug reinforcer and the other for non-drug reinforcer (ie. saline). The fixed ratio schedule gradually escalated as responses stabilized. The animals were then put on a progressive ratio challenge to measure motivation. Data may confirm an alteration in WFA and PV+ expression following intoxication or abstinence.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.09/AA4

Topic: H.08. Drugs of Abuse and Addiction

Support: R21DA058805

Title: A novel role of the mitochondrial ubiquitin ligase, MITOL, in the orbitofrontal cortex in incubation of oxycodone craving

Authors: *C. L. MATHESON[1,2], X. LUO[1], M. BURKE[1], P. COUSENS[1,2], E. MEADOWS[1], R. CHANDRA[3], X. LI[1];
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Abstract: High relapse rates are a major challenge in treating chronic opioid use, including oxycodone, a commonly abused prescription opioid. In rats, oxycodone seeking progressively increases during abstinence, a phenomenon known as incubation of oxycodone craving. We previously found that the orbitofrontal cortex (OFC) plays a causal role in the incubation of oxycodone craving, but the molecular mechanisms underlying this incubation are largely unknown. Here, we focused on the mitochondrial ubiquitin ligase, MITOL (also known as March5). MITOL is an E3 ubiquitin ligase that ubiquitinates key proteins regulating mitochondrial dynamics to target them for degradation, such as dynamin-related protein 1 (Drp1), a primary fission mediator. Preliminary work from our lab showed that mRNA expression of MITOL significantly decreased on abstinence day 15 (but not day 1) in the OFC of

rats that self-administered oxycodone compared with saline. These data suggest that MITOL in the OFC may play a negative role in incubation of oxycodone craving. To test this, we first injected an adeno-associated virus (AAV) expressing MITOL under a ubiquitous promoter (AAV2-CMV-MITOL-GFP) or AAV2-CMV-GFP (control) bilaterally into the OFC of male and female rats. Next, we trained rats to self-administer oxycodone (0.1 mg/kg, 6 h/d) for 10 days and then tested rats for oxycodone seeking on abstinence days 1 and 15. Immediately following day 15 seeking test, we collected OFC tissue for subsequent validation of March5 overexpression by using real-time polymerase chain reaction (RT-qPCR). We found that overexpressing MITOL in the OFC significantly increased both oxycodone intake during self-administration and incubated oxycodone seeking on abstinence day 15, but not non-incubated seeking on abstinence day 1, compared with the control group. Together, these findings suggest that while MITOL mRNA decreases in OFC during incubation of oxycodone craving, its function may increase during abstinence, which drives incubated Oxy seeking. Following up on these findings, current studies are probing how MITOL protein expression in OFC changes during incubation of oxycodone craving.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

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Topic: H.08. Drugs of Abuse and Addiction

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Title: Hugs or Heroin? Investigating Ventral Pallidal Cell-types in Social vs. Drug Choice

Authors: *E. RAMIREZ[1], S. WALAWALKAR[1], R. ROKERYA[1], C. SUMARINAS[1], A. GERMAIN[3], E. ARELLANO[1], S. V. MAHLER[2];
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Abstract: Opioid addiction is a major societal problem, and we still have few tools available to prevent or treat it in those who suffer—especially when it comes to tools derived from understanding of the basic neurobiology of addiction. Part of the reason for this failure to translate neuroscience findings into the addiction clinic might be due to a lack of translationally-relevant models of opioid addiction that incorporate complexities of the disorder, such as social

factors that can protect or exacerbate addiction, and which are rarely studied in preclinical studies. Indeed, social factors play key roles in the initiation and progression of addiction, and the COVID-19 pandemic laid this bare, with marked changes in drug use, abuse, overdose, and addiction occurring in the early 2020s. Here we address this knowledge gap by asking how GABA neurons in the ventral pallidum (VP), a brain region playing a key role in food and drug reward, are involved in social reward, and social modulation of drug reward. In transgenic GAD1-Cre transgenic Long Evans male and female rats, we chemogenetically inhibited or stimulated VP GABA neurons during social behaviors, and during an operant social/drug self-administration task where rats are asked to choose between social and drug rewards. Specifically, we targeted inhibitory and excitatory DREADDs (hM4Di and hM3Dq) to VP GABA neurons, allowing bidirectional control of them during behavior. Wildtype and DREADD-expressing animals were socially isolated from their same-sex, sibling cage-mate for one week before being undergoing assessments of 1) social conditioned place preference, 2) social play and investigation, 3) social communication via ultrasonic vocalizations, 4) operant social contact self-administration, and 5) choice of social vs heroin reward. Additional experiments using c-Fos immunohistology and fiber photometry were conducted to assess VP GABA and non-GABA neuronal activity during social behaviors. Our findings demonstrate that chemogenetic manipulations of VP GABA neurons modulate social reward seeking, social behaviors, and communication in rats, and do so in a domain- and sex-specific manner. These results highlight the underappreciated role of the VP in social behaviors, and takes steps toward understanding how social factors modulate opioid drug seeking in addiction more generally. It is our hope that by untangling the neural circuit substrates involving VP that impact social and drug rewards, we may develop new neural intervention strategies for leveraging social factors in preventing or treating addiction.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.11/AA6

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA grant R01 DA055849

Title: Distinct contributions of ventral pallidal GABAergic neurons to opioid and food seeking in transgenic GAD1:cre rats

Authors: *G. M. BUTCHER[1], E. RAMIREZ[2], C. RUIZ[3], M. FARRELL[4], M. X. MARTINEZ[1], E. CASTILLO[5], S. V. MAHLER[2];
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Abstract: Addiction is a heterogeneous disorder involving multiple behavioral and psychological processes. Fortunately, animal models allow for precise dissection of these processes and the specific circuit(s) supporting each. One critical circuit involves VP^{GABA} neurons, which are known to play a critical role in reward, for example by encoding reward value, motivating reward seeking, and promoting relapse. However, the specific contributions of these neurons to diverse aspects of reward-related behaviors with relevance to addiction remain incompletely understood. Here, we systematically investigated the role of VP^{GABA} in opioid drug- and food-motivated behavior in transgenic GAD1:Cre rats. We assessed the effects of reversible chemogenetic inhibition with inhibitory DREADDs (hM4Di) on a comprehensive battery of behavioral paradigms. We found that inhibition of VP^{GABA} neurons selectively impacted reward-seeking behaviors, with notable differences observed between seeking for two distinct opioid drugs, heroin and remifentanil. Specifically, we examined effects on low and high-effort opioid and food seeking, opioid versus food choice, behavioral economic measures of reward demand elasticity, and reward pursuit under various operant schedules and timeout conditions. We also assessed the impact of VP^{GABA} inhibition on anxiety-like and locomotor behaviors. Our findings reveal a previously underappreciated selectivity of VP^{GABA} neuron roles in reward. We find a critical role for them in motivated seeking of food and opioids, with a surprising lack of necessity for seeking of remifentanil. These results underscore the importance of employing diverse and complementary behavioral tasks to dissect the nuanced neural underpinnings of addiction-related behaviors. This work contributes to a more refined understanding of VP^{GABA} function and may inform the development of more targeted therapeutic strategies for opioid use disorder.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.12/AA7

Topic: H.08. Drugs of Abuse and Addiction

Title: Adult Behavioral Adaptations Following Adolescent Sucrose Exposure are Specific to Cocaine Relapse-Related Measures

Authors: *K. F. HIGDON[1,2], A. L. GARCIA LOPEZ[2], L. M. STRAND[1], X. LI[3], D. R. FEDERICO[3], N. AMISSAH[1], K. REYES[1], N. I. AHMED[4], I. S. MAZE[5,4], A. M. GANCARZ-KAUSCH[6], D. M. DIETZ[2,1,3];
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Abstract: Adolescence marks a critical period of development, accompanied by neurobiological adaptations in response to environmental exposures. Sucrose is one such factor that is ubiquitous in the modern Western diet and is consumed disproportionately by adolescents. Previously, we have demonstrated that in male rats, early life (PND 25-39) exposure to sucrose results in an enhanced vulnerability to addiction-like behaviors in adulthood (>PND60). However, given the large body of evidence of sex differences in response to both natural reinforcers and substances such as cocaine, we have recently utilized female rats to examine how this early life exposure influences various adult behaviors. Adolescent sucrose exposure attenuates cocaine seeking and responses for cues previously associated with cocaine in adulthood at both acute (AD1) and prolonged (AD30) forced abstinence periods in female rats, contrasting with previous lab data in male rats. These findings indicate the relative salience of both cues and cocaine is diminished among sucrose-exposed female rats, but did not change motivation for cocaine at either abstinence time point. To examine the role of sucrose exposure on cue salience, we performed Pavlovian Conditioning, and found that adolescent sucrose exposure did not alter goal or sign tracking, suggesting specificity for drug-associated cues. Finally, sucrose exposure did not alter motivation for other natural reinforcers, including social self-administration. To understand the neurobiological plasticity underlying behavioral alterations unique to sucrose-exposed rats, we leveraged Spatial Transcriptomics to identify transcriptional alterations in discrete brain regions, including prefrontal cortices, nucleus accumbens, and dorsal striatum. Taken together, our data demonstrate a unique adult cocaine behavioral plasticity in female rats following adolescent sucrose exposure.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR085.13/AA8

Topic: H.08. Drugs of Abuse and Addiction

Title: Association between perception of stress and psychoactive substance use

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Abstract: INTRODUCTION The use of psychoactive substances is a public health problem that has accompanied human beings since their origins. The critical age of consumption is between 18 and 34 years of age, a range that includes university students, who for various reasons are a vulnerable population for substance use. In medical students this problem is aggravated by the stressful situations that the degree intrinsically involves. Academic stress has been found to be a risk factor for the use and abuse of psychoactive substances, playing an important role in the onset, development and risk of relapse, since it generates long-term changes in various brain circuits, mediated by the hypothalamic-pituitary-adrenal axis, involving cortisol and catecholamines, which lead to a state of vulnerability, weakening the hippocampus and prefrontal cortex. AIM Association of the consumption of psychoactive substances with the perception of stress in students of the Bachelor's Degree of Medicine of an Institution of Higher Studies from July-August 2024. METHODOLOGY Descriptive, cross-sectional, observational and statistical study, conducted in a Higher Education Institution from July-August 2024, in medical students from 2nd to 9th semester. An online survey was applied to determine the frequency of psychoactive substance use and the perception of stress, to associate the variables, Pearson's Correlation Test in Graph Pad ® ver.10 was used. RESULTS A statistically significant correlation was found between the perception of stress and the consumption of psychoactive substances in medical students, since an index value of r=0.99 to 1.00 and a value of ***p<0.001 was found. Therefore, it is concluded that students consume psychoactive substances in stressful periods, so it is important to create effective strategies for stress management, thus avoiding the consideration of substance consumption as a means of stress release.

Disclosures: M. Perez Perez: None. **A. Osorio-Espinoza:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.14/AA9

Topic: H.08. Drugs of Abuse and Addiction

Support: P50 DA044118
R01 MH132680
R01 DA055849
U01 DA053826
F99NS141399
NSF GRFP DGE-1839285

Title: Adolescent THC Enhances Adult Opioid Vulnerability and Induces Prefrontal and Amygdala Transcriptional Changes

Authors: *M. X. MARTINEZ, C. M. RUIZ, A. A. CASTANEDA, C. R. PERRONE, V. C. INSHISHIAN, E. CASTILLO, H. RAMIREZ, V. SWARUP, S. V. MAHLER;
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Abstract: Adolescence is a critical period for brain development, characterized by synaptic pruning, circuit maturation, and changes in neurotransmitter systems. The prefrontal cortex and amygdala are key regions involved in decision making, cognitive control, emotional regulation, and motivation, and their adolescent development may be especially vulnerable to disruption by use of drugs like Δ^9-tetrahydrocannabinol (THC). Preclinical research has shown that adolescent THC exposure (AdoTHC) can alter cognitive function in a persistent manner, and potentially enhance susceptibility to the addictive effects of "harder" drugs like opioids.
Here we employed our well-characterized rat model of AdoTHC exposure to determine how AdoTHC persistently alters opioid drug-induced behaviors, neural activity, and gene expression. We exposed female and male Long-Evans rats to THC (5mg/kg i.p.) or vehicle (VEH) daily during adolescence (postnatal day 30 to 43). After they grew into adulthood (PD70+), rats underwent multiple behavioral tasks measuring addiction-relevant responses to opioid drugs, including heroin conditioned place preference, heroin locomotor sensitization, and behavioral economic assessments of opioid self-administration (using the highly reinforcing opioid drug remifentanil). We also assessed heroin-induced neural activity with c-Fos expression analysis in prefrontal, striatal, and amygdala brain regions, and we conducted bulk tissue RNA-sequencing in PFC and basolateral amygdala following AdoTHC/VEH, and adulthood heroin/saline (administered non-contingently twice daily in a dependence-inducing, escalating dose protocol (1, 3, 5, and 8 mg/kg, i.p., twice daily for 4 days)).
AdoTHC exposure altered opioid reward in both sexes, with especially pronounced enhancements of opioid reward and seeking in females. This sex-dependent pro-opioid phenotype was accompanied by altered brain activity in response to acute heroin in AdoTHC rats, an effect that was also distinct in males and females. Finally, gene expression patterns in PFC and basolateral amygdala of females was markedly impacted by both AdoTHC history and adulthood heroin exposure, and AdoTHC history also altered transcriptional changes induced by heroin, including in gene networks linked to immune signaling, cellular damage and repair, and neuromodulation. These findings align with prior studies reporting enhanced opioid vulnerability following AdoTHC exposure, and provide novel insights into the molecular alterations that may underlie these long-term behavioral effects.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.15/AA10

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA P50 DA044118

Title: Re-wired for Risk? Investigating the Imprint of Adolescent Cannabis Exposure on Reward and Threat Processing

Authors: *S. DIRIK[1], M. X. MARTINEZ[2], K. A. LAWSON[2], C. M. RUIZ[2], S. V. MAHLER[2]; [2]Neurobio. and Behavior, [1]Univ. of California, Irvine, Irvine, CA

Abstract: With increased accessibility, public attitudes on cannabis use are shifting through a growing perception that it is safer than other drugs. However, human correlational studies suggest a relationship between early adolescent cannabis use and long-term cognitive outcomes, including incidence of psychiatric disorders like depression, addiction, and anxiety. Preclinical studies of adolescent THC exposure (AdoTHC) also consistently report long-lasting brain and behavioral changes, including alterations in cognition, emotional regulation, and motivation-related processes. This suggests that AdoTHC may alter the adolescent development of circuits underlying reward and threat processing, and top-down regulation thereof. Since these are the same circuits thought to underlie maladaptive decision making characteristic of several psychiatric disorders, we aimed here to further characterize the specific psychological processes impacted by AdoTHC, and the altered neural substrates responsible for them.
We subjected male and female rats to our established, well-characterized AdoTHC treatment protocol, consisting of daily THC injections (IP; 0 or 5mg/kg) from postnatal days 30-43; corresponding with early-to-mid adolescence. Following a THC washout period of 21+ days, rats are trained in a series of behavioral tasks aimed at quantifying reward seeking, risk avoidance, and pursuit of reward in the face of danger. We also map c-Fos expression in several reward- and threat-related brain regions including nucleus accumbens, prefrontal cortex, amygdala, and paraventricular nucleus of the thalamus. Ultimately, these findings may shed light on whether and how adolescent THC use causally impacts behavioral and brain processes related to psychiatric disease.

Disclosures: S. Dirik: None. **M.X. Martinez:** None. **K.A. Lawson:** None. **C.M. Ruiz:** None. **S.V. Mahler:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR085.16/AA11

Topic: H.08. Drugs of Abuse and Addiction

Support: R00DA041350

Title: Role of dorsal striatal RASD1, a potential downstream target of HDAC5, in incubation of methamphetamine craving.

Authors: *P. M. COUSENS[1], M. A. M. BURKE[2], C. L. MATHESON[2], E. R. MEADOWS[2], X. LUO[2], A. T. BELEW[3], A. HUSSEY[4], R. CHANDRA[5], N. M. EL-SAYED[3], X. LI[2]; [2]Dept. of Psychology, [3]Dept. of Cell Biol. and Mol. Genet., [4]Advanced Genomic Technologies Core of the Brain and Behavior Inst., [1]Univ. of Maryland, College Park, MD; [5]Dept. of Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Addiction is a chronic relapsing disorder characterized by prolonged drug craving during abstinence and a high probability of relapse upon exposure to drug-associated cues. In rats, cue-induced methamphetamine (Meth) seeking progressively increases throughout abstinence, a phenomenon termed “incubation of Meth craving.” Our previous work demonstrates that dorsal striatal histone deacetylase 5 (HDAC5), an epigenetic enzyme that generally suppresses gene transcription, plays a critical role in the incubation of Meth craving. Specifically, we found HDAC5 overexpression and knockdown in the dorsal striatum (DS) increases and decreases Meth seeking after prolonged abstinence, respectively. Here, we aimed to identify the downstream targets of HDAC5 that underlie its role in Meth craving. We first injected adeno-associated viruses (AAVs) to either overexpress or knockdown HDAC5 in the DS of male rats. Next, we trained rats to self-administer Meth (0.1 mg/kg, 9 h/d) for 10 days. On abstinence day 35, we collected DS tissue for subsequent bulk RNA-sequencing (RNA-seq). RNA-seq data revealed that, during incubation of Meth craving, mRNA expression of dexamethasone-inducible Ras protein 1 (*Rasd1*) was decreased after HDAC5 overexpression but increased after HDAC5 knockdown in the DS on abstinence day 35, suggesting that *Rasd1* may be a transcriptomic target of HDAC5. As a member of the Ras superfamily of small GTPases, RASD1 disrupts the cAMP-PKA-CREB signaling pathway by inhibiting adenylyl cyclase. Based on the RNA-seq data, we hypothesize that RASD1, a potential downstream target of HDAC5, plays a negative role in incubation of Meth craving. Ongoing studies are investigating the effect of RASD1 overexpression in the DS on the incubation of Meth craving.

Disclosures: P.M. Cousens: None. **M.A.M. Burke:** None. **C.L. matheson:** None. **E.R. Meadows:** None. **X. Luo:** None. **A.T. belew:** None. **A. Hussey:** None. **R. Chandra:** None. **N.M. El-Sayed:** None. **X. Li:** None.

Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: PSTR085.17/AA12

Topic: H.08. Drugs of Abuse and Addiction

Support: R01DA058143
R01NS071122-07A1
R21NS133384

Title: Sex-specific impact of prenatal methamphetamine exposure on the development of the juvenile dopaminergic system

Authors: *E. J. MILLER, L. PHAN, M. CHAO, B. SANADA, A. JOSEPH, A. BUITRAGO, A. DHULIPALA, S. CAMPOS, A. GOPINATH, J. SEIBOLD, M. LIN, H. KHOSHBOUEI; Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Methamphetamine (METH) use by pregnant women is a significant and growing concern in the United States. Prenatal METH exposure is linked to various developmental issues in children (e.g., impaired intellectual functioning and emotional regulation), particularly males. However, the underlying molecular mechanisms are still largely unknown. We hypothesize that dysfunction in neuroimmune interactions between dopaminergic (DAergic) neurons and microglia during critical development periods lead to altered maturation of striatal circuitry, thereby affecting dopamine (DA) transmission in offspring exposed to METH prenatally. Pregnant dams were administered 5 mg/kg (s.c.) of METH daily until parturition. Juvenile (P20-25) offspring brains were collected for immunofluorescent staining of DAergic neurons and microglia or biochemical analysis of DA and DA metabolites. Offspring injected on P1 with a fluorescent DA sensor (pAAV-syn-dLight1.3b, i.c.v.) were reserved for ex vivo slice imaging experiments at the same timepoint. Slice recordings of dynamic dLight activity were conducted following bath perfusion of METH at a low (1uM) or high (10uM) concentration in the presence or absence of DA transporter (DAT) blockade (10uM nomifensine) or D2R antagonism (10uM sulpiride). We found that juvenile males, but not females, exposed to METH prenatally show increased expression of tyrosine hydroxylase (TH) in the striatum, indicative of altered DA transmission. Interestingly, striatal microglia from offspring with prenatal METH exposure, regardless of sex, show more activated morphology and reduced overlap with TH+ axons, suggesting potential deficits in microglial immune responsiveness and/or support in DAergic circuit maturation. While this could contribute to the etiology of increased TH+ innervation in males, females likely have alternate mechanisms to overcome this consequence of prenatal METH exposure, although additional studies are needed. Ongoing experiments are evaluating DA release in response to pharmacological manipulation of DAT or D2R dopamine receptors, with initial data in prenatally METH exposed males suggesting an increased response to METH. Future studies will focus on the age-specific developmental impact, assessing whether these effects persist through adolescence and adulthood and influence the sensitivity to the addictive potential of psychostimulants in later life. Ultimately, we hope this work will uncover the molecular implications of prenatal METH exposure on offspring DAergic pathway maturation and how disruptions in development of these circuits confer increased susceptibility to neuropsychiatric disease.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

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Program #/Poster #: PSTR085.18/AA13

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH DA055105

Title: Methamphetamine Exposure Interacts with Age to Modulate Expression of Dopamine Receptor mRNA in Parvalbumin Expressing Inhibitory Interneurons in the female rat mPFC

Authors: *A. S. BRINKS[1], E. HOFFMAN[2], L. K. CARRICA[3], J. M. GULLEY[4], J. M. JURASKA[5];
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Abstract: Exposure to psychostimulant drugs during the adolescent period has been associated with deficits in inhibition and alterations to dopamine signaling within the medial prefrontal cortex (mPFC). This may be due to disruption of normative maturation of inhibitory interneurons which occurs during adolescence. In particular, interneurons positive for parvalbumin (PV+) experience extensive development during adolescence leading to increased output. Previous work from our laboratories found that exposure to METH during early adolescence (P30-38) and adulthood (P60-68) increased the number of PV+ interneurons whereas exposure during late adolescence (P40-48) decreased the number of PV+ interneurons in the female but not male mPFC (Brinks et al, 2024). To further understand the potential underpinnings of the effects of METH on PV+ neurons, we assessed dopamine receptor expression in PV+ interneurons in the mPFC of intact female Sprague Dawley rats following exposure to METH from P30-38, P40-48, or P60-68. 24-hours following the final exposure the mPFC was collected and fluorescence *in situ* hybridization (FISH) was performed to label mRNA for *drd1*, *drd2*, and *pvalb*. We found that there were larger increases in *drd1* and *drd2* expression due to METH exposure in the adolescent period than adults. In addition, we found drd2 expression in *pvalb*+ neuron increases between early and late adolescence. We were also interested in the potential interaction between female pubertal onset and METH exposure to create the sex- and age-specific effects found previously. Thus we performed ovariectomies (OVX) at P20, prior to pubertal onset and the initiation of METH dosing. We hypothesized that OVX females would not experience significant changes to the number of PV+ neurons, similar to males in our previous study. We found that pre-pubertal OVX reduced the influence of METH on the number of PV+ neurons in the late adolescent and adult groups but not the early adolescent females.

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Poster

PSTR085: Addictive Drugs: Developmental and Behavioral Effects, Dependence, and Mechanisms

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BAW25-8106460533
CIC-UMSNH 18099
CIC-UMSNH 18146

Title: Pronociceptive effects of acute xylene and benzene exposure: Participation of 5-HT3 receptors

Authors: D. A. SIMON[1], L. F. ORTEGA-VARELA[2], D. GODINEZ-HERNANDEZ[3], C. J. GUTIERREZ-GARCIA[4], *M. Y. GAUTHEREAU-TORRES[1];
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Abstract: Xylene and benzene are misused solvents, and they are administered through inhalation to alter awareness. They are found in commercial products such as paint removers, paint thinner, gasoline, and glue, among others. Previous studies have demonstrated that abused solvents share several actions with CNS depressants, such as ethanol. Solvents have been shown to interact with ion channels, such as NMDA, GABA, and serotonergic receptors. Serotonin is linked to various functions in the nervous system, including its association with pain. Toluene (a misused solvent with a chemical structure similar to xylene and benzene) has been reported to have pronociceptive effects, acting on 5-HT_3 serotonergic receptors. However, the role of other abused solvents in nociception or whether other solvents share the effects of toluene is unknown. The aim of this study was to evaluate the effect of acute xylene and benzene exposure on nociception, as well as the involvement of serotonergic 5-HT_3 receptors. Female Wistar rats were administered subcutaneously with m-CPBG (serotonergic 5-HT_3 agonist), alosetron (serotonergic 5-HT_3 antagonist) or saline (vehicle), 10 min before acute exposure to xylene (6000 ppm) or benzene (8000 ppm) or air during 30 minutes. Subsequently, rats were administered subcutaneously with 50 µl of 1% or 0.5% formalin. Acute nociception was assessed for 1 min every 5 min for 1 h. Data are expressed as the mean ± S.E.M. of 6 animals per group. Our results showed that acute exposure to xylene and benzene enhances formalin-induced acute nociception. Local peripheral pretreatment with alosetron (10-100 nmol) reduced the pronociceptive effect of benzene, but not of xylene, on acute nociception. Furthermore, local peripheral administration of m-CPBG (300 nmol) increased acute nociception induced by 0.5% formalin in rats exposed to air and benzene, but not to xylene. Our findings suggest that xylene

and benzene have a pronociceptive effect, like toluene, and 5-HT_3 receptors are involved in benzene effect.

Disclosures: D.A. Simon: None. **L.F. Ortega-Varela:** None. **D. Godinez-Hernandez:** None. **C.J. Gutierrez-Garcia:** None. **M.Y. Gauthereau-Torres:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

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Program #/Poster #: PSTR086.01/AA15

Topic: H.08. Drugs of Abuse and Addiction

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RO1NS071122-07A1
R21NS13384

Title: Craving Without Dopamine? Sex Differences in Methamphetamine-Associated Cue Conditioning

Authors: *L. PHAN, E. MILLER, A. GOPINATH, D. BORG, A. NEWMAN, S. KRAMER, J. SEIBOLD, M. LIN, H. KHOSHBOUEI;
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Abstract: Methamphetamine (METH) Use Disorder (MUD) remains a significant public health crisis, imposing substantial burdens on society, the economy, and healthcare systems. The psychostimulant METH hijacks the brain's reward system, dramatically increasing extracellular dopamine levels through multiple mechanisms. While MUD affects both men and women, biological factors such as sex differences remain poorly understood. Notably, female METH users are at greater risk of developing dependence and experiencing higher relapse rates, even when using similar quantities of METH. Additionally, females are more likely to self-administer low doses of METH, whereas males tend to self-administer higher doses. Despite these differences, most studies primarily focus on male models of MUD, limiting our understanding of sex-specific neurobiological responses. We hypothesized that female mice exhibit greater dopamine release in a METH-paired environment compared to male mice. To test this hypothesis, we measured dopamine release dynamics in the ventral striatum of freely behaving male and female C57Bl/6 mice equipped with GRIN lens in the in the ventral striatum. Four weeks after lens implantation and AAV-αSyn-dLight1.3b injection in the ventral striatum, animals underwent conditioned place preference (CPP). Over eight days, animals received alternating injections of METH (2 mg/kg, i.p.) and saline (i.p.). Simultaneous in vivo imaging and behavioral assessments were performed during drug-paired conditioning and preference test days. Repeated METH exposure during conditioning phase significantly increased dopamine levels in the ventral striatum of both males and females mice ($n=5$, $p<0.05$). The METH-induced increased striatal dopamine was associated with heightened psychomotor responses in both

sexes, with females displaying greater sensitivity despite receiving a similar dose of METH (2mg/kg) compared to males. Contrary to our hypothesis, preliminary data revealed that although both male and female mice exhibited CPP to the drug-paired side neither males nor females exhibited increased dopamine peak frequencies for METH-associated place preference. These findings suggest that increased METH-associated contextual cues are attributed to non-dopaminergic transmission, such as GABAergic or glutamatergic signaling from medium spiny neurons within the ventral striatum. These findings also highlight fundamental sex differences for METH-associated conditioning providing critical insights into the neurobiological mechanisms underlying MUD and the development of sex-specific prevention and treatment strategies.

Disclosures: L. Phan: None. **E. Miller:** None. **A. Gopinath:** None. **D. Borg:** None. **A. Newman:** None. **S. Kramer:** None. **J. Seibold:** None. **M. Lin:** None. **H. Khoshbouei:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

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Topic: H.08. Drugs of Abuse and Addiction

Support: National Science and Technology Council (NSTC)

Title: Exploration of the effects and neural mechanisms of environmental enrichment in inhibiting methamphetamine-induced sensitized behavior

Authors: *C.-N. CHENG[1], A. C.-W. HUANG[2];
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Abstract: This study mainly investigated whether different types of environmental enrichment (EE) play a key role in regulating behavioral sensitization and anxiety symptoms induced by methamphetamine (MAMPH). To this end, we designed a variety of housing conditions, including standard housing without enrichment (SH), standard enriched housing with full enrichment (STEE), physical enrichment with emphasis on physical activities (PEE), cognitive enrichment with learning stimulation (CEE), and social enrichment with emphasis on peer interaction (SEE). In the experiment, mice were randomly assigned to different housing and injection combinations, including SH with saline, SH with MAMPH, and four types of EE with MAMPH. The dose of MAMPH was 1 mg/kg per day for seven consecutive days, and saline was uniformly injected on the eighth day. Behavioral tests and c-Fos immunohistochemical staining analysis were then performed to evaluate changes in neural activity in specific brain regions. The results showed that MAMPH could effectively induce behavioral sensitization, but had no significant effect on anxiety behavior. It is worth noting that among all kinds of enrichment conditions, STEE was the most effective in inhibiting behavioral sensitization caused by

MAMPH, while the effects of PEE, CEE and SEE were relatively limited. Further c-Fos analysis indicated that the medial prefrontal cortex (including Cg1, PrL and IL), nucleus accumbens (NAc), basolateral amygdala (BLA), ventral tegmental area (VTA), caudate putamen (CPu) and CA1 and CA3 regions of the hippocampus were all involved in this sensitization process. Among them, STEE had a significant regulatory effect on the neural activity of these regions, especially in Cg1, IL, NAc, BLA, VTA, CPu, CA3 and dentate gyrus (DG). Overall, the results show that specific types of environmental enrichment, especially fully integrated STEE, may provide an effective non-drug intervention strategy for reducing behavioral sensitization caused by MAMPH, which has potential significance for understanding and developing alternative therapies.

Disclosures: C. Cheng: None. **A.C. Huang:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant R00DA049719
SUNY Research Foundation

Title: Midbrain Neuronal Ensembles Activated During Methamphetamine Seeking Behavior

Authors: *I. R. GAWANDE[1], E. RAKOWSKI[2], S. DOMINGUEZ-LOPEZ[3];
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Abstract: The dopamine mesolimbic system modulates the reinforcing and rewarding effects of methamphetamine. However, methamphetamine chronic users develop deficiencies in dopamine-related brain functions that become more severe with increased exposure and consumption of the drug. We previously reported that mice chronically exposed to methamphetamine self-administration showed signs of an increased oxidative environment of the ventral mesolimbic dopamine pathway. In addition, we found that there is an increase in dopamine neurons firing at lower rates, accompanied by irregular bursting activity in the ventral tegmental area (VTA). Whether this change in dopamine neuronal activity is involved in methamphetamine seeking behavior is unknown. Here, we will present a study on neuronal ensembles activated by methamphetamine-seeking behavior in mice chronically exposed to methamphetamine self-administration. We crossbreed FosTRAP mice with an inducible Cre-dependent TdTomato (TDT) reporter strain. This mouse model enables the genetic tagging of neuronal populations that express c-Fos within an activated brain circuit. We implanted FosTRAP/TDT adult mice with a jugular catheter and trained them daily to nose poke for methamphetamine infusions (0.1

mg/kg/infusion). After training, mice were placed in a fixed ratio 3 (FR3) schedule to complete up to 3-4 weeks of methamphetamine self-administration exposure. Then, mice were administered 4-OH tamoxifen (50 mg/kg) and exposed to an extinction session to TRAP neuronal ensembles during seeking behavior. We used tyrosine hydroxylase (TH) immunolabeling on brain slices to locate neuronal ensembles in dopamine mesolimbic regions activated during methamphetamine-seeking behavior. Brain slices were then registered against the Allen Mouse Atlas to enumerate TRAPed ensembles by brain region. Our preliminary results indicated that mice exposed to methamphetamine self-administration for several weeks recruited additional brain circuits outside the mesolimbic dopamine pathway during methamphetamine seeking. In our initial comprehensive midbrain mapping, we found that TRAPed ensembles in the whole midbrain region included TH+ and TH- neurons in motor and behaviorally related areas. Surprisingly, in the VTA and substantia nigra, most TRAPed neurons were not TH+. Our study provides supporting evidence of brain circuits outside the dopamine mesolimbic system encoding drug-seeking behavior after exposure to methamphetamine self-administration over several weeks.

Disclosures: I.R. Gawande: None. **E. Rakowski:** None. **S. Dominguez-Lopez:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant GM149450-2
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Title: Effects of nicotine on microglia activation and methamphetamine reward in adolescent rats

Authors: A. LUJAN, A. MORA, M. SANCHEZ, A. MARTINEZ, A. ESPINOSA, *C. A. CRAWFORD;
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Abstract: Cigarette smoking and vaping during adolescence are common and lead to several long-term adverse consequences. Preclinical studies have shown that adolescent rats are more sensitive to the rewarding effects of nicotine and that those exposed to nicotine are more responsive to illicit drug administration, including methamphetamine, in adulthood. This heightened sensitivity to methamphetamine following adolescent nicotine exposure may result from increased neuroinflammation. Specifically, nicotine enhances microglial activation during adolescence, and this activation is now recognized as an essential contributor to addictive behavior. In this study, we utilized conditioned place preference (CPP) to assess whether adolescent nicotine amplifies the acquisition of methamphetamine-induced place preference. We

also investigated the impact of inflammation by measuring whether rats treated with nicotine during adolescence exhibit greater expression of the inflammatory marker Iba-1 in the nucleus accumbens. Male and female Sprague-Dawley rats were injected with nicotine (0, 0.16, 0.32, or 0.64 mg/kg, ip) once daily for 20 consecutive days, beginning on postnatal day (PD) 25. On PD 45, methamphetamine-induced CPP was evaluated using a 10-day biased CPP procedure, which included one preconditioning day, eight conditioning days (comprising alternating daily injections of saline or MA (0 or 1 mg/kg, ip), and one test day. Twenty-four hours after the test day, rats were perfused, and Iba-1 expression was evaluated. Overall, female rats had greater place preference scores, regardless of nicotine treatment, than male rats. However, nicotine (0.64 mg/kg) exposure enhanced MA-induced CPP in both males and females. Rats that received methamphetamine had more Iba-1-positive cells than those receiving saline. Nicotine treatment slightly intensified this response in male rats but not in females. These results further illustrate that adolescent nicotine treatment boosts the rewarding value of methamphetamine, although the mechanism behind this enhancement may differ between male and female rats.

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Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

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Program #/Poster #: PSTR086.05/BB3

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant R03DA054461

Title: Lack of sex differences in methamphetamine and sucrose self-administration when comparing rats from the same behavioral groups

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Abstract: Rationale: We recently published a report revealing that there were no sex differences in cocaine-induced locomotor activity when we compared male and female rats that belonged to the same behavioral group(s) using our new MISSING (Mapping Intrinsic Sex Similarities as an Integral quality of Normalized Groups) model. However, it is not known if this model would also be relevant for a different behavior (psychostimulant self-administration) in a different rat strain (Long Evans rats, LE rats). Our hypothesis was that there are no sex differences in methamphetamine (METH) and sucrose self-administration when we compare male and females LE rats that belong to different behavioral groups.
Methods: We allowed male (n = 14) and female (n = 26) LE rats to self-administer METH (0.1 mg/kg/infusion, FR1, 6h per day, 5 days per week for 4 weeks). For natural reinforcer control

group, we allowed male (n = 16) and female (n = 16) LE rats to self-administer sucrose (1-20mg pellet, FR1, 6h per day, 5 days per week for 4 weeks). We obtained variables for 1) baseline intake (defined as drug intake prior to attainment of the plateau phase; the plateau phase was estimated for non-linear regression analysis of the intake-time curve using sigmoidal function as curve fitting model), 2) total intake (defined as summation of number of drug infusions over the 4 weeks), and 3) total intake_nba (nba = normalized to baseline intake activity and time) which was calculated as total intake/ (baseline × N, where N = time for total intake/ time for baseline intake). To identify distinct behavioral groups, we employed normal mixtures clustering analysis of these 3 variables. To determine that the groups were distinct, we plotted a 3-D graph of the relationships between these 3 variables for each identified cluster and compared this structure for all clusters. We did this also for groups categorized using median split of total intake and using biological sex. For the MISSING model, we employed 2-way ANOVA with factors being SEX (males, females) and groups (clusters).
Results: From BIC analysis, our clustering model identified groups that were more distinct from each other than the groups identified via median split or biological sex. For the METH study, we identified 3 behavioral groups via clustering. The MISSING model revealed no SEX by cluster interaction and there were no sex differences within each cluster. For the sucrose study, we identified 3 behavioral clusters, and the MISSING model also revealed no sex differences within each cluster.
Conclusions: There are no sex differences in METH and sucrose self-administration when we compare males and females that belong to the same behavioral group(s).

Disclosures: I. Madhuranthakam: None. **M. Job:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.06/BB4

Topic: H.08. Drugs of Abuse and Addiction

Support: P20GM130461

Title: Activation of lateral hypothalamic melanin-concentrating hormone neurons mediates sex-specific patterns of methamphetamine self-administration in rats.

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Abstract: Sensitivity to the reinforcing effects of methamphetamine (METH) can influence the motivation to take the drug; this in turn can contribute to the development of METH use disorder (MUD). Though the neural systems underlying differences in methamphetamine reinforcement, especially between sexes, are poorly understood several brain areas are known to play a role in

regulating METH associated behaviors. The lateral hypothalamus (LH) interfaces with mesolimbic neural circuitry associated with reward and MUD. Within the LH, melanin-concentrating hormone (MCH) neurons, mainly studied in the context of sleep wakefulness and energy homeostasis, have been shown to impact METH psychomotor sensitization. These and other findings suggest that LH MCH neurons regulate METH seeking and taking behavior, but relatively little is known about how the activity of these neurons influence METH self-administration behavior. In this study, female and male Wistar rats trained to intravenously self-administer METH (0.05 mg/kg/inf) via nose pokes for 10 days. After this, a dose-response curve was generated by allowing rats to self-administer one of five doses (0.0625, 0.0125, 0.025, 0.05, or 0.1 mg/kg/inf, every other day. Using a combinatorial viral approach, we targeted activating designer receptors exclusively activated by designer drugs (DREADDs) to MCH-positive LH neurons. On test days, 30 minutes before self-administration sessions, rats received saline control or clozapine-n-oxide (CNO, 0.3 or 1 mg/kg) in order to activate MCH neurons. Female and male rats readily self-administered METH and a dose response curve was generated where the lowest dose resulted in the most nose pokes, and the highest dose resulted in the fewest. Stable responding to the training dose was seen in between dose response days. MCH activation produced bidirectional, sex-specific effects: enhanced methamphetamine intake in female and reduced intake in male rats, particularly at lower methamphetamine doses. Despite equivalent acquisition rates between sexes during training, divergent behavioral outcomes emerged only after targeted MCH activation. These findings suggest that MCH signaling does not exert uniform effects on reinforcement behavior but instead regulates the sensitivity to methamphetamine's rewarding properties in a sex- and methamphetamine dose-dependent manner. These findings underscore the critical role of hypothalamic MCH neurons in modulating drug reward and highlight sex as a necessary biological variable in future pharmacotherapeutic strategies for psychostimulant use disorders.

Disclosures: M. Suarez: None. **I.R.K. Kuebler:** None. **C.E. Bass:** None. **K.T. Wakabayashi:** None.

Poster

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Program #/Poster #: PSTR086.07/BB5

Topic: H.08. Drugs of Abuse and Addiction

Support: NSF 1922428

Title: Trpm8 null mutant mice exhibit altered reward: sucrose, ethanol and amphetamine

Authors: E. B. SALDES[1], A. ERDMIER[2], S. ASUTHKAR[2], ***T. E. KOELTZOW**[4,3];
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Abstract: The Transient Receptor Potential Melastatin 8 (TRPM8) is a Ca++ permeable ion channel that is a high affinity target for testosterone. We've previously reported that TRPM8 null mutant mice exhibit increased sexual behavior and aggression (Mohandass et al., 2020), and transcriptonomic analyses revealed that deletion of the TRPM8 protein elicits upregulation of genes relevant to addiction in reward-relevant regions of the brain (Saldes et al., 2025). Presently, we report the results of immunohistochemical analyses indicating that TRPM8 is expressed by midbrain dopamine neurons, suggesting that TRPM8 may play a critical role in reward. To test this hypothesis, male and female wildtype (C57BL/6J) and TRPM8 -/- mutant mice (Jackson Laboratories, Bar Harbor, ME) were subjected to a two-bottle sucrose preference procedure (n= 6-7/condition). Whereas no differences were observed at low sucrose concentrations (0.1 and 0.5%) TRPM8 null mutant mice exhibited a statistically significant increase in preference for 1% sucrose solution compared to regular drinking water. This effect was observed in both male and female mice. Similarly, male TRPM8 -/- mice exhibited a statistically significant increase in the preference for 3% and 10% ethanol compared to wildtype mice. Among female mice, no statistically significant differences were observed at the lower concentration whereas 10% ethanol appeared to elicit an aversion in wildtype mice. Finally, mice were subjected to a sensitizing regimen of amphetamine: On Day 1, mice were administered i.p. injections of 1.0 mg/kg amphetamine (Sigma Aldrich, St. Louis, MO) followed by 3.0 mg/kg amphetamine on Days 2-6. On Day 7, a sensitization test was performed using 1.0 mg/kg amphetamine. Each day, mice were given 20 minutes to habituate to the test chambers, followed by a 20-minute saline challenge prior to amphetamine testing, which concluded after 70 additional minutes. Across all measures of locomotor activity, female mice were less active compared to males. Both male and female TRPM8 (-/-) mice exhibited an attenuated response to amphetamine compared to wildtype mice. Taken together, these results indicate that deletion of the TRPM8 protein alters the response to natural reward and to substances of abuse. Despite the ability of testosterone to rapidly and powerfully activate the TRPM8 channel, no meaningful sex differences were observed.

Disclosures: E.B. Saldes: None. **A. Erdmier:** None. **S. Asuthkar:** None. **T.E. Koeltzow:** None.

Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: 1R01DA056484-01A

Title: Anaerobic bacteria and amphetamine use disorder: Bacterial biofilms as key regulators of drug response

Authors: C. GONZALEZ-ARANCIBIA[1], S. NAKKELLA[1], Y. ZHU[1], A. SUNIL KUMAR[2], A. GALLI[3], A. M. CARTER[4];
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Abstract: Rich microbial diversity in the gut microbiome is associated with healthy host physiology while dysbiosis is correlated with a range of pathologies, including neurological disorders. Congruently, antibiotic administration can affect multiple aspects of behavior, including behaviors governed by the dopaminergic system. Antibiotics reduce amphetamine (AMPH)-induced behaviors and extracellular dopamine (DA) levels in rodents, suggesting that microbes could play a role in modulating host responses to psychostimulants. Previous data from our lab showed that *Fusobacterium nucleatum (F. nucleatum)* gut colonization of fruit flies (*Drosophila melanogaster)* enhances AMPH-associated behaviors such as locomotion and courting. Consistent with these behaviors being regulated by DA signaling, we also found that *F. nucleatum* enhances AMPH-induced DA efflux in brains of flies. Interestingly, AMPH abuse also alters the microbiome, including increasing the abundance of *F. nucleatum* in both rodents and humans. *F. nucleatum* is an anaerobic bacteria found primarily in the oral cavity and, in lower amounts, throughout the gastrointestinal tract. How AMPH enhances *F. nucleatum* gut's abundance is unknown. We found that AMPH did not affect the growth of *F. nucleatum* cultures but instead increased biofilm formation. *F. nucleatum* is thought to form biofilms in order to establish an oxygen-free niche to facilitate colonization of oxygenated environments. RNA sequencing and qRT-PCR revealed that AMPH up-regulates *fap2*, *radD*, and *fadA* gene expression in *F. nucleatum.* These genes encode adhesion proteins which facilitate the attachment of *F. nucleatum* to surrounding surfaces and other bacteria. We also found that mutant bacteria lacking the *fap2* gene (Δfap2) do not form biofilms, even in the presence of AMPH, and treatment of *F. nucleatum* cultures with D-Galactose, a competitive inhibitor of Fap2, decreases AMPH-enhanced biofilm formation. These data suggest that Fap2 is required for AMPH enhancement of *F. nucleatum*'s biofilm formation. Additionally, flies colonized with the Δfap2 mutant did not showed AMPH-enhanced DA efflux, nor AMPH-enhanced locomotion, suggesting that Fap2 is required for *F. nucleatum* enhancement of AMPH-induced dopaminergic signaling. We propose a biofilm-dependent feed-forward paradigm for AMPH, *F. nucleatum*, and host responses to AMPH, where AMPH increases gut's abundance of *F. nucleatum* by regulating adhesin proteins and biofilm formation, and *F. nucleatum* enhances AMPH effects in the host by regulating DA signaling.

Disclosures: C. Gonzalez-Arancibia: None. **S. Nakkella:** None. **Y. Zhu:** None. **A. Sunil Kumar:** None. **A. Galli:** None. **A.M. Carter:** None.

Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: RJM (R01MH122844)

Title: Effects of Chronic Cannabis Use on Stress-induced Neural and Immune Responses in Rats

Authors: *A. T. ATTAH, Z. FISHER, R. KONSPORE, C. EASTMAN, D. LINDENFELSER, R. J. MCLAUGHLIN;
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Abstract: Stress, a modulator of the immune response is a major reason for chronic cannabis use. Studies have shown that chronic cannabis use dampens stress-induced neuroendocrine response in humans and rodents. However, the central mechanisms subserving chronic cannabis-induced alterations in the stress response, and its downstream impacts on stress-induced immune responses are unknown. Thus, we investigated the effects of chronic cannabis vapor self-administration on 1) stress-induced activation of neurons expressing corticotropin-releasing factor type-1 receptors (CRFR1), and 2) recruitment of pro- and anti-inflammatory cytokines in male and female CRFR1-Cre:tdTom rats that express Cre-recombinase and a fluorescent reporter (tdTomato) in neurons that express CRFR1. Rats were trained to self-administer vaporized cannabis extract (150 mg/ml cannabis in PEG-400) or vehicle (PEG-400 only) in daily one-hour sessions for 30 days. On day 31, all rats were acutely restrained for 30 minutes. Ninety minutes after stress termination, rats were euthanized for the collection of brain and cardiac blood. Brains were cryo-sectioned and stained for c-fos immunoreactivity to assess activation of CRFR1 neurons in the mPFC, BNST, PVN, CeA, BLA, MeA, PVT, and CPu. Neurons expressing c-fos, and CRFR1-expressing neurons colocalized with c-fos were counted and analyzed using 2-way ANOVA. Plasma levels of the cytokines IL-1β, IL-6, IL-10, and TNF-α; were measured using ELISA and analyzed using 2-way ANOVA. Chronic cannabis vapor self-administration significantly decreased c-Fos reactivity in CRFR1-expressing neurons in the mPFC of acutely stressed rats compared to the vehicle group ($p < 0.05$). However, no significant effects were observed in other brain regions. Plasma cytokine analysis revealed a significant reduction in IL-10 levels in acutely stressed male (but not female) rats exposed to cannabis vapor compared to vehicle ($p < 0.05$), which suggests attenuated stress-induced recruitment of anti-inflammatory pathways in male rats. IL-1β, IL-6, and TNF-α; levels remained unchanged across all groups. These findings suggest that chronic cannabis vapor self-administration selectively dampens stress-induced activation of CRFR1-expressing neurons in the mPFC and impairs anti-inflammatory immune responses in males, highlighting a potential mechanism by which chronic cannabis use may dysregulate neuroimmune interactions under stress and raising important considerations for sex-specific health outcomes associated with long-term cannabis use.

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Poster

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Program #/Poster #: PSTR086.10/BB8

Topic: H.08. Drugs of Abuse and Addiction

Title: Pharmacokinetic profiling of Δ9-THC metabolism and its association with cognitive impairment and modulation by stress

Authors: *T. M. MYERS[1], M. M. TORREGROSSA[2];
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Abstract: The use of cannabis has been increasing in recent years due to its legalization and decriminalization, and the growing use of cannabis for medicinal purposes. However, while cannabis can have positive effects in some individuals, there are potential negative consequences including cannabis dependence, psychosis, and cognitive impairments. Individuals often report using cannabis to alleviate stress, however, whether or not cannabis differentially induces positive or negative effects under conditions of stress has not been well studied. Developing a deeper understanding here will allow for better individualized decisions to be made on the medicinal use of cannabis. The current study investigates whether stress affects performance on a working memory task, and if Δ9-THC (THC), the primary psychoactive component in cannabis, can exacerbate or ameliorate the effects of stress. An additional aim of this study is to determine if THC concentrations in the blood are associated with cognitive performance. 24 adult male and female Sprague Dawley rats were trained to perform a three choice, delay-match-to-sample working memory task. Rats assigned to the stress group were exposed to 45 minutes of restraint stress prior to injection of either vehicle, 0.5,1, or 3 mg/kg THC. Blood samples were collected 5, 25, 60, and 120 minutes after administration. A repeated-measures ANOVA revealed an effect of delay in the males ($p < 0.0001$) and females ($p < 0.0001$) on working memory performance, with no effect of stress or THC. However, 3 mg/kg THC disrupted responsivity on the working memory task, leading to a subset of rats exhibiting non-responsiveness. Further analysis revealed that the non-responders showed elevated THC plasma levels 5 minutes post 3mg/kg THC administration relative to responders ($p < 0.0001$). Additionally, non-responders showed greater THC metabolite levels. Overall, plasma concentrations of THC and its metabolites may predict levels of sensitivity to THC. Future directions include examining the relationship between stress, CB1R binding and working memory performance.

Disclosures: T.M. Myers: None. **M.M. Torregrossa:** None.

Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: R01 DA052859 (DWW)
U01 DA058527 (DWW)

Title: Cannabidiol and cocaine induce heterogenous and donor-dependent inflammatory responses in primary human peripheral immune cells

Authors: *R. COLÓN-ORTIZ[1], S. KNERLER[2], L. MAMUN[2], J. ROSADO-FRANCO[2], D. W. WILLIAMS[2];
[1]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Pharmacol. and Chem. Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: While the immunomodulatory potential of common recreational drugs has been largely evaluated in resident immune cells of the central nervous system (CNS), emerging studies suggest that the peripheral immune system also contributes to neuroimmune interactions during CNS pathology in neuropsychiatric and degenerative disorders, including addiction. Peripheral immune cells, like monocytes, macrophages, and T cells, become activated during immune perturbations and are recruited to the brain across the blood brain barrier (BBB), where they secrete pro- or anti-inflammatory cytokines. Further, these cells express receptors for neurotransmitters that are intrinsically involved in perpetuating molecular pathways of addiction, such as cannabinoid (CBRs) and dopamine receptors; indicating a more complex yet poorly understood contribution of the peripheral immune system during substance abuse. Our lab previously showed that both cannabidiol (CBD) and cocaine (COC), which are widespread drugs known for their effects on the CNS, exhibit immunomodulatory properties during neuroinflammatory insults. Whether these drugs play a role in immune processes that may help treat or prolong reward-addiction pathways remains unknown. We hypothesized that CBD attenuates, while COC exacerbates, inflammatory responses in peripheral monocytes and T cells that direct immune cell recruitment to the brain during substance abuse. To begin to test this, we cultured primary human monocytes, macrophages, and T cells from blood donors with CBD, the CBR agonist CB-13, or COC for 24 hours. LPS was used as a positive control. After, cells and supernatants were collected for bulk RNA-seq and LEGENDPlex multiplex bead-based cytokine analysis. Surprisingly, we found large inter-donor variability in cytokine induction in response to both CBD and COC, which suggests that the immunomodulatory effects of these drugs occur in a donor-specific manner. Interestingly, CB-13 suppressed LPS-induced cytokines, reflecting CBD's anti-inflammatory potential during immune perturbation. In the future, evaluating the direct impact of CBD and COC on immune cell recruitment at the BBB may reveal trends in neuroimmune interactions that mechanistically contribute to heterogenous immune responses during substance abuse.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA T32 DA007234
P50 DA037844
NIDA R01 DA056485

Title: Cause and affect: indicators and consequences of THC-seeking behavior in heterogeneous stock rats

Authors: *A. MASKERI[1], S. M. SPENCER[2];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Pharmacol., Univ. of Minnesota, Lino Lakes, MN

Abstract: Cannabis use disorder (CUD), characterized by repeated cannabis use in spite of psychological and social consequences, has increased among adults following the implementation of recreational and medical cannabis laws across 39 states. With the user base of cannabis and related products increasing each year, it is imperative to better understand individual responses and how they inform future risk. The operant behavioral model of drug self-administration, extinction, and cue-induced reinstatement emulates cue priming and drug craving in patients in recovery experiencing settings, cues, or memories associated with past drug use. Innate reward processing, anxiety-like behaviors, and initial drug response all predict future substance-seeking in cocaine and opioid contexts and have potential to do so in a cannabinoid setting. Additionally, tolerance to cannabinoid administration develops rapidly and can alter both reward processing and pharmacological effects as well as baseline anxiety levels. To further elucidate these interactions, we chose to study cannabis seeking in a heterogeneous stock rat strain characterized by a wide range of traits. Previous work has established a rodent model of THC self-administration and preliminary data collection also reveals discrete and sex-dependent anxiety responses in addition to differential reward processing patterns and pharmacological responses to acute cannabinoid administration. The goals of this study are to repurpose established metrics of reward seeking and pharmacological response as potential predictors of cannabinoid use, and to investigate how repeated cannabinoid exposure can alter these same metrics over time. Insight gained from these experiments will narrow the knowledge gap in cannabinoid research while addressing limitations in current human and animal literature.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA DA000523-17

Title: Structure-activity relationships for MDMB-4en-PINACA and related analogs to induce cannabinoid-like effects in C57Bl/6J mice

Authors: *N. R. GONZALEZ, A. D. MAITLAND, M. H. BAUMANN, G. C. GLATFELTER; Designer Drug Res. Unit, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Synthetic cannabinoid receptor agonists (SCRA) are novel psychoactive substances designed to mimic the effects of delta-9-tetrahydrocannabinol (THC) through the activation of cannabinoid-type 1 receptors (CB1). SCRAs are often more potent and efficacious than THC, leading to hospitalizations, overdose, and in rare cases, fatalities. Among the currently available SCRAs, MDMB-4en-PINACA has been the most frequently detected compound in toxicological case work in recent years. The current study compared the cannabinoid-like effects of MDMB-4en-PINACA and related analogs—MMB-4en-PINACA and 5F-MDMB-PINACA—to the reference SCRA, AM-2201. For dose-response experiments, groups of male and female C57Bl/6J mice (n=12 per drug; 6 female) received subcutaneous (s.c.) SCRA (0.001-3 mg/kg) or vehicle (5% DMSO: 5% Tween-80: 90% Saline) injections. Behavioral effects were evaluated using a test battery which measures body temperature changes, catalepsy (bar test), and antinociception effects (52℃ hot plate test) every 30 min over a 2-h session relative to baseline measurements. Temperature was monitored via a s.c. implanted transponder that emits signals to a handheld recorder. Separate experiments assessed the role of CB1 receptors using rimonabant pretreatment (0.5 mg/kg, s.c.) 15 minutes prior to SCRA treatment and triad testing. All of the SCRAs produced dose-related cannabinoid-like effects, including hypothermia, catalepsy, and antinociception that were significantly different from vehicle ($p<0.05$). 5F-MDMB-PINACA had the highest potency (ED50=0.01-0.04 mg/kg) and produced the greatest degree of hypothermia (temp. change= -9℃). These cannabinoid-like effects were attenuated when pretreated with a rimonabant antagonist. From a structure-activity perspective, compounds with the MDMB head moiety exhibited higher potencies than their MMB analogs. Our findings reveal how structural modification of SCRA head groups can markedly influence potency and efficacy for inducing in vivo effects.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: Supported by Texas Tech University Health Sciences Center Jerry H. Hodge School of Pharmacy Department of Pharmaceutical Sciences Startup Funding.

Title: Sex dependent effects of CBD, Beta caryophyllene and other cannabis-derived terpenes on nociception and body temperature in mice

Authors: *L. KHODAVIRDILOU, J. WILKERSON;
Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX

Abstract: Cannabidiol (CBD) is widely studied for its analgesic and anti-inflammatory properties, while cannabis-derived terpenes are proposed to modulate cannabinoid effects through the "entourage effect." This study investigated the antinociceptive and thermoregulatory effects of CBD, beta-caryophyllene (BCP), alpha-terpineol (ATP), and gamma-terpinene (GTP) in male and female C57BL/6J mice using a cumulative intraperitoneal dosing protocol (56-320 mg/kg). Antinociception was assessed with the hot plate test (expressed as % Maximum Possible Effect, %MPE), and thermoregulation was evaluated via rectal temperature measurements (ΔT). CBD-induced dose-dependent antinociception and hypothermia in male mice, with no analgesic effects in females. BCP and GTP also elicited antinociceptive responses in males only, while ATP showed no analgesic activity in either sex. All compounds induced dose-dependent hypothermia, with BCP demonstrating the most robust and consistent effect across sexes. In combination with CBD, BCP enhanced both antinociception and hypothermia in males. ATP+CBD produced modest analgesia, while GTP+CBD reduced %MPE compared to CBD alone, suggesting antagonism. None of the combinations produced significant antinociceptive or thermoregulatory changes in females. These findings underscore the importance of sex as a biological variable in cannabinoid pharmacology and identify BCP as a promising candidate for enhancing CBD-mediated analgesia, particularly in males.

Disclosures: L. Khodavirdilou: None. **J. Wilkerson:** None.

Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA IRP - Z1A DA000633

Title: Neuronal versus glial CB2 receptor: Findings from a new strain of CB2-knockout and CB2-eGFP reporter mice

Authors: E. LINZ[1], H. ZHANG[3], F. RUBIO[4], Q.-R. LIU[5], ***Z. XI**[2];
[1]Mol. Targets and Medication Discovery, [2]Natl. Inst. on Drug Abuse, Baltimore, MD; [3]Natl. Inst. of Mental Hlth., Bethesda, MD; [4]Behavioral Neurosci. Res., NIDA IRP, Baltimore, MD; [5]Lab. of Clin. Investigation, Natl. Inst. on Aging, Baltimore, MD

Abstract: The expression of CB2 receptors (CB2Rs) in neurons versus glial cells remains debated. While CB2R expression is generally associated with microglia, direct evidence is limited and inconsistent. In CB2R-GFPTg mice (Schmöle et al., 2015), GFP signals were detected in microglia, but no GFP signal was observed in CB2-eGFP-flox mice (Lopez et al., 2018). These discrepancies may stem from differences in GFP knock-in strategy. In Schmöle's

strain, the GFP was randomly inserted into mouse genome via BAC, with unknown genomic integration sites. In Lopez's strain, eGFP was inserted into the 3′-UTR of the CB2 gene with flanking loxP sites, which may not reflect endogenous CB2 promoter activity. To address this issue, we generated a new strain of CB2-KO-eGFP mice by replacing the CB2-coding sequence with GFP while preserving surrounding genomic structures. qRT-PCR and RNAscope confirmed loss of CB2 expression. Immunostaining revealed eGFP expression in the cortex, hippocampus, red nucleus, and VTA in CB2-KO-eGFP but not wild-type mice. Co-labeling showed eGFP in TH^+ dopaminergic neurons in the midbrain. Fluorescence-activated cell sorting (FACS) showed ~4% of $NeuN^+$ neurons and ~70% of $Ki67^+$ microglial cells expressed eGFP in CB2-KO-eGFP cortex and hippocampus, compared to ~0.5% and <20% in WT mice. Behaviorally, CB2-KO-eGFP mice exhibited greater locomotor activity and increased body weight from 6 months of age onward, without altered nociceptive responses. In conclusion, this novel CB2-KO-eGFP mouse line may serve as a full CB2-KO and a reporter of endogenous CB2 expression in both neurons and microglia, offering a valuable model to study CB2R function in the brain.

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Poster

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Topic: H.08. Drugs of Abuse and Addiction

Support: T32 AG061892
R01 AG072714

Title: Chronic Oral Delta-9-THC Consumption Increases Impulsive Decision Making in Aged Rats

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Abstract: Background: With the rise in cannabis use among older adults, it is important to understand its effects on the unique cognitive and neural profiles in this age group. Older adults tend to prefer large, delayed over small, immediate rewards (i.e., reduced "impulsive choice"); however, how cannabis affects this pattern in aging remains unclear. To address this gap, we used aged rats to evaluate how both acute and chronic oral delta-9-tetrahydrocannabinol (THC, the primary psychoactive component of cannabis) influence intertemporal decision making in aged rats. Methods: Fischer 344 x Brown Norway F1 hybrid rats (n=19, 9F, 10M) were trained to choose between a small, immediate food reward and a large food reward delivered after variable delays. For acute testing, each rat consumed 0, 0.3, and 1.0 mg/kg THC gelatin in a randomized order thirty minutes before behavioral testing. Following a one-week washout

period, chronic effects were assessed by having rats consume either control or 1.0 mg/kg THC gelatin daily for four weeks. To preclude acute THC effects on behavior, intertemporal choice testing occurred in the morning, with gelatin administration in the afternoon. Task performance (percentage of large reward choices) was analyzed via repeated measures ANOVA. Results: Acute THC administration had no effect on intertemporal choice performance, with no main effect of dose ($p = 0.83$) or interaction effects. In contrast, chronic THC reduced preference for the large, delayed reward after 4 weeks, while controls showed stable performance across the same timeframe (Week × Delay × Drug condition interaction; $F(4,52) = 2.84$, $p = 0.033$), indicating increased impulsive choice following chronic THC consumption. Conclusions: These findings highlight the potential impact of chronic THC use on decision-making and impulsivity in aging populations, emphasizing the need for further research to better understand cognitive and behavioral effects of cannabis use in older adults. Ongoing work is using fiber photometry during task performance to investigate how chronic THC affects dopamine release in the basolateral amygdala, a brain region important for intertemporal decision making.

Disclosures: E. Gazarov: None. **S. Pattey:** None. **J.L. Bizon:** None. **B. Setlow:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.17/BB15

Topic: H.08. Drugs of Abuse and Addiction

Support: University of Saint Thomas Psychology Department

Title: Cannabis use, academic burnout, and physiological responses to a cognitive task

Authors: S. EBSEN[1], G. TUPA[1], S. JOHNSON[1], ***M. GADES**[2];
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Abstract: Background: Cannabis is the most widely used psychoactive substance among college students, which is concerning since this population is also at an elevated risk of burnout due to intense course work. Burnout is related to lower feelings of accomplishment, higher stress levels, and poorer academic achievement, making its management crucial for wellbeing and academic performance. Past research yields inconsistent results, but several studies found associations between cannabis use and decreased motivation and reward sensitivity. There is also evidence that cannabis impacts cognition, including decreased brain activation during cognitive tasks. Decreased motivation and cognitive performance associated with cannabis may contribute to academic burnout. This study, therefore, examined the relation between cannabis use, burnout, and physiological stress responses. Methods: Participants were undergraduate students (N=38) recruited from the University of St. Thomas, who were at least 18 years old. Participants completed the Burnout Assessment Tool to measure feelings of mental exhaustion; the GAD-7 and PHQ-9 to measure anxiety and depression symptoms; and self-reported frequency of

cannabis use. In a lab setting, participants completed three levels of the Dual N-back task to measure working memory and also to induce stress. Biopac sensors measured physiological stress, specifically heart rate and electrodermal activity (EDA), at baseline and during the task. Blood pressure was measured before and after the task. T-tests compared burnout scores and physiological measures between cannabis current-users and nonusers. Results: There were no significant differences in self-reported burnout, anxiety, or depression between cannabis users and non-users. Performance on level two of the N-back was significantly poorer for users than nonusers (t=2.283, p=.015). The percentage change in heart rate was significantly different between groups, slightly increasing for nonusers and decreasing for users during the task (t=2.249, p=.016). EDA and blood pressure did not differ between the groups. Conclusions: As cannabis use is becoming increasingly popular and legalized in many states, it is important to understand implications of using. Results can inform future research on cannabis and its effects in academic settings. The decrease in heart rate for users may indicate less motivation to perform well on the task compared to non-users, making it feel less stressful. Poorer N-back performance for users may suggest negative cognitive effects of cannabis use. This information can help students make informed decisions about cannabis use.

Disclosures: S. Ebsen: None. **G. Tupa:** None. **S. Johnson:** None. **M. Gades:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.18/CC1

Topic: H.08. Drugs of Abuse and Addiction

Support: California Department of Cannabis Control Grant 93301 (PI: Natasha Wade)
R01DA054106 (PI: Joanna Jacobus)
K08 DA050779 (PI: Natasha Wade)
T32 AA013525 (PI: Riley/Spadoni)

Title: Blood Plasma Cannabinoids and Cannabis Use Disorder Status Predict Poorer Working Memory Function

Authors: *V. SZPAK[1], J. JACOBUS[2], V. THUMAR[2], E. L. DELFEL[1], A. L. WALLACE[2], N. E. WADE[2];
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Abstract: Background: Research shows an association between heavy cannabis use in young adults and disruptions in neurocognitive performance that are subserved by the dorsolateral prefrontal cortex (dlPFC). Variability in cannabinoid products (e.g., potency, route of administration) may influence measurement of use and assessment of downstream relationships

within the dlPFC. Further multimodal research is needed to assess these associations. This study includes objective (plasma cannabinoid concentration), subjective (self-reported cannabis use problems and other substance use), and neurocognitive testing to examine brain-behavior relationships in young adults who use cannabis. **Methods:** Participants (N=103) aged 18-20 years old were included from a larger study examining the effects of cannabis and nicotine co-use. Blood plasma samples quantified 11-nor-9-carboxy-tetrahydrocannabinol (THCCOOH) concentration, the primary metabolite of Δ9-tetrahydrocannabinol (THC). Three separate linear regression models were conducted to examine the effects of plasma THCCOOH concentration, cannabis use disorder (CUD) status, and their interaction on cognitive performance. Outcomes included attention and working memory (RAVLT Trial 1), inhibitory control (NIH Toolbox Flanker task), and processing speed (NIH Toolbox Pattern Comparison task). All models controlled for sex assigned at birth and past 30-day alcohol and nicotine use. **Results**: Plasma THCCOOH concentration ($\beta = -0.66$, $p = 0.04$), CUD status ($\beta = -1.05$, $p = 0.006$), past 30-day nicotine use ($\beta = -0.06$, $p = 0.008$) were significant predictors of poorer working memory performance (RAVLT Trial 1). There was a significant interaction between THCCOOH and CUD status ($\beta = 0.67$, $p = 0.04$). Follow-up analyses revealed that individuals without CUD who had higher THCCOOH concentrations exhibited poorer RAVLT Trial 1 performance compared to individuals with CUD. No relationships with inhibitory control or processing speed were detected. **Discussion**: Results suggest that performance with the cognitive domains of attention and working memory may be vulnerable to heavy cannabis use for those without CUD. This could be due to changes in neuroanatomical regions associated with these domains. Given that performance in individuals with CUD appear less affected by higher THCCOOH concentration, this could reflect their tolerance or neuroadaptation to heavy cannabis use. Overall, this indicates that cognitive deficits from cannabis use may be underestimated in those without CUD. **Conclusions**: This study highlights that the relationship between THCCOOH concentration and working memory may vary by CUD status.

Disclosures: V. Szpak: None. **J. Jacobus:** None. **V. Thumar:** None. **E.L. Delfel:** None. **A.L. Wallace:** None. **N.E. Wade:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.19/CC2

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant P20GM113109

Title: Effects of voluntary ingestion of synthetic delta-9-tetrahydrocannabinol on the hedonic value of rewarding and aversive substances and CB1 expression

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Abstract: Human studies indicate a relationship between Δ-9-tetrahydrocannabinol (THC) and alcohol use, yet research on their co-dependency remains limited. Rodent models have shown that cannabinoids can enhance the incentive salience and hedonic value of sucrose and alcohol. However, results seem to vary by the functions of THC dose and route of administration. The present study addresses these discrepancies by using a translational model of THC consumption, using lower oral doses of synthetic THC (Dronabinol). Male Long-Evans (n = 24) were surgically implanted with an intraoral fistula. Following recovery, rats consumed a vehicle or THC-containing cookie (0.05 mg/kg or 0.5 mg/kg) before testing the palatability of rewarding and aversive substances in the taste reactivity paradigm. Separate, linear, mixed-effect models revealed that THC dose dependently increased hedonic reactions to both sucrose (0.1M & 0.5M) and alcohol (10% & 40% ETOH) compared to control. Comparatively, THC reduced aversion to alcohol (10% & 40%) and quinine compared to vehicle. Western blot data analyzed via one-way ANOVA followed by Tukey's post-hoc analysis revealed that repeated oral THC consumption dose dependently downregulated cannabinoid receptor 1 expression in the dorsal hippocampus, while no significant changes were observed in the nucleus accumbens or amygdala. These findings suggest that oral THC enhances the hedonic value of rewarding and aversive substances and provide insights into the neurobiological mechanisms that may contribute to the bidirectional use of cannabis and alcohol.

Disclosures: D. Laux: None. **B. Waymire:** None. **T. Polton:** None. **M. Cain:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.20/CC3

Topic: H.08. Drugs of Abuse and Addiction

Support: NIDA Grant 1U01DA058278

Title: Prenatal delta-9-tetrahydrocannabinol exposure induces limbic transcriptomic dysregulation and isoform shifts in developing brain

Authors: *Y. LEE, W. CHEN, Z. CHEN, C. WANG;
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Abstract: Cannabis is the most widely used illicit drug during pregnancy, with studies reporting increases ranging from 60% to over threefold over the past two decades. Prenatal cannabinoid exposure (PCE) has been linked to an increased risk of neurodevelopmental and addiction-like disorders. However, the underlying molecular mechanisms remain poorly understood. Here, using a well-established PCE rat model, we showed that PCE led to increased hyperactivity in 3-week-old offspring, which was more prominent in male offspring. To identify key genes and biological processes in the limbic-cortical pathway following PCE, we employed RNA sequencing to analyze transcriptomic alterations and alternative splicing in the nucleus

accumbens (NAc) obtained from postnatal day 7 (P7) offspring brains after PCE. Additionally, spatial transcriptomics was carried out on the medial prefrontal cortex (mPFC) tissues obtained from postnatal day 24 (P24) offspring brains after PCE. Principal component analysis (PCA) of RNA-seq data revealed clear separation between PCE and control in both male and female. We identified 63 differentially expressed genes (DEGs) in male and 353 DEGs female in NAc due to PCE. The gene ontology (GO) terms were enriched for neurodevelopment and neurotransmitter transport. Pathway analysis of DEGs showed enrichment in addiction-related pathways in both sexes. Furthermore, differential transcript usage (DTU) analysis revealed isoform shifts in genes related to axon development, CNS patterning, and neurotransmitter transport. Additional analysis of splicing alterations, including distal polyadenylation site usage and cell-type enrichment patterns, was performed. In parallel, spatial transcriptomic profiling of the P24 male mPFC revealed gene expression changes in PCE rats related to energy metabolism and toxic substance response, as evidenced by the altered expression of glutamate-ammonia ligase, suggesting potential metabolic disruption and glutamate toxicity. These findings demonstrate that PCE leads to transcriptomic and behavioral changes during early brain development, highlighting the potential pathways through which *in utero* cannabis exposure may alter neurodevelopment.

Disclosures: Y. Lee: None. **W. Chen:** None. **Z. Chen:** None. **C. Wang:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.21/Web Only

Topic: H.08. Drugs of Abuse and Addiction

Title: Involvement of hippocampal D1-like dopamine receptor in the effect of cannabidiol on extinction and reinstatement of methamphetamine -induced CPP

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Abstract: Methamphetamine (METH) is a major health problem without effective pharmacological treatment. Cannabidiol (CBD), a component of the Cannabis sativa plant, is believed to have the potential to inhibit drug-related behavior. However, the neurobiological mechanisms responsible for the effects of CBD remain unclear. Several studies have proposed that the suppressing effects of CBD on drug-seeking behaviors could be through the modulation of the dopamine system. The hippocampus (HIP) D1-like dopamine receptor (D1R) is essential for forming and retrieving drug-associated memory. Therefore, the present study aimed to investigate the role of D1R in the hippocampal CA1 region on the effects of CBD on the extinction and reinstatement of METH- conditioned place preference (CPP). For this purpose,

different groups of rats over a 10-day extinction period were administered different doses of intra-CA1 SCH23390 (0.25, 1, or 4 μg/0.5 μl, Saline) as a D1R antagonist before ICV injection of CBD (10 μg/5 μl, DMSO12%). In addition, a different set of animals received intra-CA1 SCH23390 (0.25, 1, or 4 μg/0.5 μl) before CBD injection (50 μg/5 μl) on the reinstatement day. The results revealed that the highest dose of SCH23390 (4 μg) significantly reduced the accelerating effects of CBD on the extinction of METH-CPP ($P < 0.01$). Furthermore, SCH23390 (1 and 4 μg) in the reinstatement phase notably reversed the preventive effects of CBD on the reinstatement of drug-seeking behavior ($P < 0.05$ and $P < 0.001$, respectively). In conclusion, the current study revealed that CBD made a shorter extinction period and suppressed METH reinstatement in part by interacting with D1-like dopamine receptors in the CA1 area of HIP.

Disclosures: R. Azizbeigi: None. **A. Farrokhi:** None. **A. Haghparast:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.22/CC4

Topic: H.08. Drugs of Abuse and Addiction

Support: DA009621 to MEW

Title: Role of nucleus accumbens core D1 and D2 medium spiny neurons and glutamate inputs from paraventricular nucleus of the thalamus in the incubation of methamphetamine craving

Authors: *G. S. DRISCOLL, E.-K. HWANG, M. M. BEUTLER, H. M. KUHN, J. G. WESTLAKE, M. E. WOLF;
Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The persistence of vulnerability to craving and relapse is a significant barrier to successful methamphetamine (meth) use disorder treatment. Neural mechanisms underlying this vulnerability can be studied using the translationally relevant ‘incubation of craving’ model, in which rats exhibit progressive intensification (incubation) of cue-induced drug craving following withdrawal from drug self-administration. Our lab previously demonstrated that incubation of meth craving is associated with upregulation of calcium permeable-AMPA receptors (CP-AMPARs) in nucleus accumbens core (NAcc) medium spiny neurons (MSN), and that NAcc CP-AMPAR activation is required for the expression of incubated meth craving. We are now focusing on how MSN subtypes (D1 or D2 receptor expressing) and glutamatergic inputs to the NAcc contribute to CP-AMPAR plasticity and incubation of meth craving. Previously, we found CP-AMPAR upregulation in D1 but not adenosine 2A (A2a) receptor-expressing MSN (A2a and D2 receptors colocalize) after meth incubation. We have therefore used chemogenetics to test the hypothesis that NAcc D1 MSN are required for the expression of incubated Meth craving. Adult male and female D1-Cre or A2a-Cre transgenic Long Evans rats received Cre-dependent AAV-

hM4Di (inhibitory DREADD) in NAcc, self-administered meth (0.1 mg/kg/infusion) under extended access conditions (6 h/d x 10 d), then received cue-induced seeking tests on withdrawal (WD) day 21 preceded by injection of the DREADD ligand J60 (0.3 mg/kg, i.p.) or vehicle. Although we use both sexes, we are not powered to detect sex differences because both sexes show meth incubation and NAcc MSN CP-AMPAR upregulation. Preliminary results (6-9 rats/group) indicate that inhibition of D1 MSNs reduced expression of meth seeking ($p = 0.05$ vs. vehicle), whereas inhibition of NAcc A2a MSNs had no effect. We are currently conducting complementary photometry studies of NAcc D1 and A2a MSN during expression of meth incubation. We are also examining the role of specific glutamatergic inputs to the NAcc in meth incubation, focusing on inputs from the paraventricular nucleus of the thalamus (PVT) based on electrophysiological evidence that PVT-D1 MSN NAcc synapses show enhanced glutamatergic synaptic transmission via upregulated CP-AMPAR and NMDAR transmission following meth withdrawal. First, chemogenetic studies are in progress to explore the functional role of PVT-NAcc pathway in expression of meth incubation by expressing AAV-hM4Di in PVT and delivering CNO to NAcc. Second, we are using photometry to measure activation of nerve terminals in NAcc originating from PVT during expression of meth incubation.

Disclosures: G.S. Driscoll: None. **E. Hwang:** None. **M.M. Beutler:** None. **H.M. Kuhn:** None. **J.G. Westlake:** None. **M.E. Wolf:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.23/CC5

Topic: H.08. Drugs of Abuse and Addiction

Support: DA009621 to MEW

Title: Incubation of methamphetamine craving is associated with cell-type specific synaptic adaptations in the rat central amygdala

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Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: A major challenge in treating methamphetamine (meth) use disorder is a high rate of relapse even after prolonged abstinence. In the 'incubation of craving' model of persistent relapse vulnerability, cue-induced drug seeking in rats progressively intensifies after withdrawal from drug self-administration (SA) and then remains high for long periods. The central nucleus of amygdala (CeA) is a brain region implicated in reward, motivation, anxiety, stress-related disorders and the reinforcing effects of drugs of abuse. It has been shown that increased activity of CeA is associated with cue-induced meth seeking during late withdrawal (after incubation), but the synaptic mechanisms underlying CeA involvement remain largely unknown. In this study, we examined synaptic adaptations during meth incubation, focusing on a subpopulation of

adenosine A2a receptor (A2a) expressing neurons in the CeA. Adult male and female Long Evans rats self-administered saline or meth under extended-access conditions and received cue-induced seeking tests on withdrawal day (WD) 1 and WD21 to establish incubation. Whole-cell patch clamp recordings were performed after WD21 (sample sizes 8-15 cells/6-8 rats). We use A2a-Cre rats (the A2aR colocalizes with the D2R) crossed with reporter rats to enable identification of the A2a expressing subtype of CeA neurons; D1-Cre rats were used to identify the D1 receptor expressing subtype. We found increased membrane excitability and excitation-inhibition balance in synaptic transmission for A2a+ CeA neurons, but not D1+ CeA neurons, after withdrawal from meth SA compared to saline controls ($p<0.05$). Interestingly, strengthened glutamatergic synaptic transmission evoked by electrical stimulation of A2a+ CeA neurons occurred through the upregulation of high conductance calcium-permeable AMPA receptors (CP-AMPAR) ($p<0.01$ vs saline controls). Additionally, A2a+ CeA neurons exhibited increased NMDA receptor-mediated synaptic responses after meth incubation ($p<0.05$). Our data suggest that abstinence from meth induces cell type-specific synaptic plasticity in the CeA and increased excitatory tone through CP-AMPAR and NMDAR upregulation in A2a+ CeA neurons. This may contribute to expression of incubated meth craving. We are currently extending these studies to explore the role of A2a+ CeA neurons in meth seeking behavior using chemogenetic approaches.

Disclosures: E. Hwang: None. **M.M. Beutler:** None. **M.E. Wolf:** None.

Poster

PSTR086: Basic Mechanisms of Drugs of Abuse

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR086.24/CC6

Topic: H.08. Drugs of Abuse and Addiction

Support: NIH Grant DA51100

Title: Monitoring In-Brain Cocaine Concentrations during Self-Administration

Authors: K. HONEYWELL[1], K. WANG[3], M. R. STOCCO[2], J. GERSON[4], ***T. E. KIPPIN**[5]; [1]Psychological and Brain Sci., [2]Univ. of California, Santa Barbara, Santa Barbara, CA; [3]Psychological & Brain Sci., Univ. of California Santa Barbara Neurosci., Santa Barbara, CA; [4]UC Santa Barbara, Santa Barbara, CA; [5]Univ. California, Santa Barbara, Santa Barbara, CA

Abstract: Cocaine addiction, overdose, and death are on the rise. Central to cocaine taking as well as explanations of cocaine taking are rapid and dynamic changes in cocaine concentrations in the brain. For instance, snorting or inhaling cocaine is more addictive than other routes and, in rats, changes of infusion times of mere tens of seconds can abolish the reinforcing properties of the drug. However, there is a dearth of detailed information on how cocaine concentrations rise and fall in relation to reinforcement particularly for individual subjects. Thus motivated, we adopted an electrochemical aptamer-based (EAB) sensor with 15 s temporal resolution, stable drift characteristics, and sub-micromolar detection limits for cocaine to the task of measuring

cocaine in-situ in the striatum of rats taking cocaine. Rats were trained to lever press for cocaine (1 mg/kg delivered intravenously) during "recreational'-type sessions of 1 hour per day for 15 days. Rats took on average 8.5 +/- 2.3 mg/kg in 1 hour sessions. Next, a cocaine sensor was placed into striatum and the rats were allowed to self-administer cocaine. Rats took a range of infusion from 5-15 during the recording session and reached peak cocaine concentrations of between 3 and 7.5 micromolar. Thus we observed stark variability in not only self-administration as typically seen but also that concentration profiles exhibit high variability. In addition, all rats continued to show rising cocaine concentrations throughout the session indicating that there is not stabilization of brain concentrations of cocaine during the first hour of intake. These results highlight the need for understanding the in brain cocaine concentration dynamics at the individual level as well as further consideration of how, if at all, cocaine concentrations are titrated during voluntary consumption.

Disclosures: K. Honeywell: None. **K. Wang:** None. **M.R. Stocco:** None. **J. Gerson:** None. **T.E. Kippin:** None.

Poster

PSTR087: Human Attention Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR087.01/CC7

Topic: I.01. Attention

Title: Thalamocortical dynamic processes underlying attention in rapid serial visual presentation

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Abstract: Introduction: Rapidly processing the visual world is a skill that requires people to deploy attention to detect relevant, often fleeting information. Prior work has suggested that dynamic changes in cortical and subcortical oscillations, particularly in the theta (3-8 Hz) and beta (13-30 Hz) bands, may be critical to attention processing, yet the precise direction of these changes remains unclear. Furthermore, middle frontal gyrus (MFG) may be an important region for reorienting endogenous attentional processes and integrating activity from the dorsal and ventral visual streams. **Methods:** Here, we employed a unique single-stream rapid serial visual presentation (RSVP) task previously developed by our group to investigate the oscillatory changes associated with activating attention to a novel target in the context of lag-1 sparing. Epilepsy patients with intracranial electrodes were asked to perform the RSVP task. Behavioral performance and corresponding local field potentials were recorded from CMT, MFG, and MTG for the duration of the task period. **Results:** Our analyses demonstrated a consistent decrease in MFG (2/3; 10/10 contacts), MTG (7/8, 18/23), and CMT (2/2 contacts) beta power from the time of cue (“T1”) presentation within the RSVP stream to approximately 200ms following cue onset on trials where the target (“T2”) was correctly identified compared to trials where T2 was

missed. This initial beta suppression was followed by a rebound in beta power 200-300ms after the cue in all three regions. The degree of beta suppression was greater in MFG and CMT compared to MTG. Furthermore, we observed an increase in theta power between 400-500ms after cue onset in CMT (2/2) and MFG (8/10). These findings were supported by a subsequent ROC analysis, which demonstrated that increased theta power in CMT and MFG during the 500ms post-cue window was more predictive of correct performance. **Conclusions:** These findings provide an initial characterization of the role of thalamocortical processing in the deployment and adaptation of attentional resources under challenging temporal constraints in the human visual system and suggest a potential relationship between the known integrative capacity of higher order visual processing areas (e.g., MFG, MTG) and thalamic oscillations.

Disclosures: A.R. Kimata: None. **L.R. Quinn:** None. **T. Watanabe:** None. **W.F. Asaad:** None.

Poster

PSTR087: Human Attention Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR087.02/CC8

Topic: I.01. Attention

Title: Rhythmic dynamics in human pulvinar and cortex during cued visuospatial attention

Authors: *E. CUNNINGHAM[1], C. LUSTIG[2], W. C. STACEY[3], D. BRANG[2];
[1]Univ. of Michigan, Evanston, IL; [2]Psychology, [3]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Rhythmic oscillations in visuospatial detection have been linked to theta-rhythmic (3-7 Hz) oscillatory activity in macaque pulvinar, contributing to theories that input from the mediodorsal pulvinar coordinates an attentional network (consisting of pulvinar, FEF, and PPC) responsible for rhythmic sampling of the environment. Here, our aim is to extend these observations to the human pulvinar, describing the relation between pulvinar and cortical oscillatory phase and visuomotor reaction time (RT) using intracranial thalamic sEEG in patients with epilepsy (N = 6). We focus on the hypothesis that the phase of pulvinar theta-rhythmic activity should be linked to periods of relatively impaired/enhanced performance, consistent with alternation between attentional engagement/disengagement at a given spatial location. Each patient performed one or more sessions of a cued visuospatial task that involved searching for a small visual target in a constantly updating array of nontarget stimuli. Although performance on the task was generally strong, evidence for theta-rhythmic phase-RT coupling within the pulvinar was mixed (and theta-rhythmic peaks in task-wide power spectra were relatively weak/unstable, compared with alpha/beta-range patterns). For most contacts, small peaks in phase-RT coupling within the 2-8Hz range were neither statistically robust nor stable across sessions. However, some evidence for robust, theta-specific phase-RT coupling was observed among the most dorsal pulvinar contacts, particularly when attention was directed toward the contralateral hemifield. In cortical contacts, theta-rhythmic oscillatory peaks and theta-band phase-RT coupling, when

present, were stronger than effects observed in the pulvinar, with a frontoparietal distribution similar to that described in previous reports (especially when attention was directed to the contralateral visual field). Taken together, these results provide preliminary evidence for theta-rhythmic phase-RT coupling in the dorsal pulvinar, consistent with evidence for rhythmic sampling from the mediodorsal pulvinar of the macaque. However, the absence of more extensive pulvinar effects and reduced SNR in pulvinar relative to cortex highlight the need for further investigation.

Disclosures: E. Cunningham: None. **C. Lustig:** None. **W.C. Stacey:** None. **D. Brang:** None.

Poster

PSTR087: Human Attention Dynamics

Location: SDCC Halls B-H

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Program #/Poster #: PSTR087.03/CC9

Topic: I.01. Attention

Support: NIH Grant P50 MH132642
NIH Grant RO1 EY017699
NIH Grant R01 MH137624

Title: The role of pulvinar in mediating cortical high-frequency activity during selective attention in human iEEG

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Abstract: Modulation of high-frequency activity (HFA) underlies many cognitive processes, in particular selective attention. Attention in general provides salient signals to investigate the larger question of what roles different brain regions play during cognitive processes, and how those brain regions interact. A particular feature of interest is that HFA, even when observed across large cortical regions, tends to be phase-locked to low-frequency rhythms, particularly in the theta (4-8 Hz) range. However, the mechanisms underlying this synchronization of HFA across distributed brain regions remain unclear, especially in the cases of cortical areas that only have weak direct connections. We hypothesize that higher-order thalamic nuclei—especially the pulvinar—have a key role in coordinating this long-range synchronization by entraining outgoing signals to separate cortical regions through theta-band oscillations.
Here, we approach this question in intracranial recordings humans. We analyzed cortical and thalamic responses from intracranial electrodes implanted in six epilepsy patients while they performed a visual spatial attention task (i.e. Egly-Driver task) where visual cues signaled the

likely location of upcoming targets. In preprocessing the data, we compared commonly used referencing approaches: closest-white-matter, bipolar montage, and local average reference. We noted that bipolar referencing has a tendency to obscure low-frequency rhythms, such as theta, as low frequencies tend to spread across contacts; we also observe closest-white-matter referencing may add noise in cases where contacts in white matter receive multiple volume-conducted signals. For this dataset, we selected a local average reference approach expanding on Laplacian referencing: referencing a subgroup of channels on each electrode shank preserved both low-frequency signals and HFA. With this preprocessing, we observed robust locking of thalamic theta to cue onset events. Furthermore, preliminary analysis also shows that HFA bursts across the cortex are phase-locked to the theta phase extracted from pulvinar contacts, indicating that higher-order thalamic nuclei are indeed involved in synchronizing HFA across cortical regions. These findings provide novel insight into the role of the thalamus in coordinating cortical activity during attention by leveraging low-frequency oscillations to facilitate inter-areal communication.

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Poster

PSTR087: Human Attention Dynamics

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR087.04/CC10

Topic: I.01. Attention

Support: University of California, Santa Barbara Academic Senate Research Grant
Alfred P Sloan Research Fellowship

Title: Distributed spatial attention does not reliably attenuate stimulus-evoked responses

Authors: *A. H. HARRISON, T. C. SPRAGUE;
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Abstract: When spatial attention is directed to multiple locations simultaneously, perceptual sensitivity is typically reduced relative to attending a single location (e.g., Ling & Carrasco, 2006; Popovkina et al., 2021). Consistent with this, neural responses are often attenuated under distributed compared to focal attention (e.g., McMains & Somers, 2005; Toffanin et al., 2009). However, in a recent fMRI study, we observed a different pattern: focal spatial attention enhances stimulus representations, but this enhancement does not decline when a second stimulus is also attended (Harrison et al., VSS 2024). Because fMRI signals may reflect top-down inputs from control regions rather than stimulus-locked sensory responses, and EEG and fMRI index distinct physiological correlates of attention (Itthipuripat et al., 2019), we conducted a follow-up EEG experiment using a selective attention task modeled on our prior fMRI design to better characterize the neural mechanisms of distributed spatial attention. On each trial,

participants were cued to attend to fixation, one peripheral location, or two peripheral locations, while flickering stimuli (20 and 24 Hz) appeared in each lower quadrant of the screen. We analyzed steady-state visually evoked potentials (SSVEPs) to quantify stimulus-driven responses across attention conditions. Attending to a single peripheral stimulus increased evoked power relative to the attend-fixation baseline, but we observed no consistent reduction in overall SSVEP amplitude when attention was distributed to both stimuli versus focused attention on one stimulus. We further examined attentional modulation in the alpha band and in time-resolved SSVEP to assess how distributed attention impacts processing dynamics. These findings align with our prior fMRI results, supporting a model in which spatial attention enhances early visual responses without a strong cost for dividing attention across locations when the task requires continuous stimulus monitoring.

Disclosures: A.H. Harrison: None. **T.C. Sprague:** None.

Poster

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Topic: I.01. Attention

Support: NSF Grant 2120539

Title: Distinct oscillatory mechanisms simultaneously support attentional sampling and distractor suppression

Authors: *Z. V. REDDING, Y. DING, I. C. FIEBELKORN;
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Abstract: Neural oscillations across multiple frequency bands influence the selective processing of visual information. Theta-band activity (~4-8 Hz) has been linked to alternating periods of attention-related sampling and shifting (i.e., to explore visual space), while alpha-band activity (~8-13 Hz) has been linked to perceptual cycles and distractor suppression. Oscillations across both frequency bands have been linked to fluctuations in neuronal excitability. Here, we used scalp-recorded EEG to test how these oscillatory mechanisms influence the processing of visual information and perceptual sensitivity during a task that cued the likely locations of both a low-contrast target and a high-contrast distractor. For trials without distractors, perceptual sensitivity (as measured with d-prime) depended on the pre-stimulus phase of theta-band activity (~7 Hz) recorded at frontocentral electrodes, consistent with theta-rhythmic attentional sampling at the cued target location. In addition to theta-band activity at frontocentral electrodes, perceptual sensitivity on no-distractor trials depended on the pre-stimulus phase of alpha-band activity (~10 Hz) at occipital electrodes. This second oscillatory process was only observed at electrodes that were contralateral to the cued distractor location. The pre-stimulus phase of occipital alpha-band activity was also associated with fluctuations in distractor-evoked ERPs, consistent with phasic

distractor suppression. Our findings thus reveal two oscillatory mechanisms that simultaneously promote attention-related sampling and distractor suppression. Comparing these mechanisms within the same behavioral task reveals their unique contributions to selective processing of visual information.

Disclosures: Z.V. Redding: None. **Y. Ding:** None. **I.C. Fiebelkorn:** None.

Poster

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Topic: I.01. Attention

Support: NSF Grant 2120539
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Title: Using rapid frequency tagging to track attentional shifting and sampling

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Abstract: Complex visual scenes contain multiple behaviorally important objects. The metaphorical spotlight of attention must therefore shift around a scene to solve behavioral goals (e.g., to find a bag of peas in the frozen vegetables section at a grocery store). Neurophysiological evidence suggests that such attentional shifting (and sampling) has a rhythmic temporal structure (at ~3-8 Hz), but continuous tracking of this spotlight across multiple objects in a visual scene can be methodologically difficult. This is particularly true for scalp-recorded EEG, given considerable volume conduction of the electrical signals. Here, we propose a novel EEG experiment that makes use of rapid frequency tagging (RFT) to deduce trial-level changes in attentional deployment across multiple objects, including the precise timing of attentional shifts. We manipulate cue validity and the number of behaviorally relevant objects to create experimental conditions when it is behaviorally advantageous to shift attention often, less often, or not at all. We then simultaneously measure the temporal dynamics of attentional modulation across multiple frequency-tagged objects. Our preliminary results have verified the feasibility of our approach, demonstrating attentional modulation of the frequency-tagged objects across different cueing conditions. We will now measure how changing the number of behaviorally relevant objects and/or changing cue validity changes the temporal dynamics of attentional shifting (and sampling). We also use a novel method for inducing RFT. Specifically, we used recently developed high refresh-rate OLED monitors, which are more accessible and affordable for EEG researchers than previously used projectors. These monitors provide a promising alternative for trial-level monitoring of attentional deployment with scalp-recorded

EEG, revealing the neurophysiological time-course of attentional deployment across diverse experimental contexts.

Disclosures: P.J. Cavanah: None. **I.C. Fiebelkorn:** None.

Poster

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Title: Neural Dynamics of Spatial-Featural Processes that Facilitate Selective Visual Attention

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Abstract: The goal of this project is to understand how the brain integrates object representations with behaviorally relevant spatial locations under different attentional conditions. Feature-based (FBA) and spatial attention (SA) are often considered independent processes, but how they dynamically interact to support goal-directed behavior remains unclear. We investigate the spatiotemporal neural dynamics of these interactions using high-density EEG during a visual search task, focusing on how attention coordinates brain networks during template formation, maintenance, and target selection. To manipulate attentional demands, we designed a visual search task with three conditions: spatial (cued location), feature (cued image category), and spatial-feature (combined cue). Naturalistic image patches from various categories were used to preserve the complexity of real-world vision and align with known properties of high-level visual cortex. Unlike prior studies that focused on low-level features (e.g., color, shape), our design examines attention's interaction with high-level representations, such as object category. Behavioral data show systematic differences in reaction time (RT). RTs were fastest for spatial trials, slowest for feature trials, and intermediate in the combined condition. These results suggest that SA enables efficient selection processes when spatial cues are sufficient, while FBA may recruit broader processing networks. Intermediate RTs in the combined condition imply complex interactions where feature-based demands may constrain attentional processes. EEG analyses revealed that alpha and theta power varied with attentional state, further supporting distinct dynamics. To test computational mechanisms, we use multivariate pattern (MVP) classifiers trained on stimulus-evoked EEG patterns from a passive viewing task using image category labels. CLIP embedding for the same images are used to model latent object structure. Classifiers are then tested on cue and delay-period activity during search trials. Above chance

decoding after cue onset and during delay would suggest that FBA reactivates and maintains high-level object representations in the absence of sensory input. Complementary representational similarity analyses compare EEG and CLIP-derived dissimilarity matrices over time to track how representations are preserved. By comparing decoding trajectories across attentional conditions, we aim to reveal how attention dynamically reactivates and transforms latent representations to guide behavior. Our approach highlights how cognitive processes may be implemented through representational dynamics.

Disclosures: J. Gonzalez-Amoretti: None. **A.C. Snyder:** None. **I.C. Fiebelkorn:** None.

Poster

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Topic: I.01. Attention

Support: P50MH132642
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R01MH137624

Title: The rhythmic nature of saccade timing in naturalistic visual search

Authors: *A. A. SCHEIBE[1,3], R. BOSHRA[1], Y. WU[1], F. BARTSCH[1], X. LIU[2], P. DE WEERD[3], S. KASTNER[1,2];
[1]Princeton Neurosci. Inst., [2]Dept. of Psychology, Princeton Univ., Princeton, NJ; [3]Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands

Abstract: The Rhythmic Theory of Attention posits that intrinsic theta rhythms (~4-8 Hz) organize neural activity into alternating attentional states: one favoring information sampling at the current focus of attention, and another promoting attentional shifts to new locations. While this framework has been primarily tested in highly controlled tasks under central fixation (i.e., during covert attention), it remains unclear whether similar rhythmic mechanisms also operate under naturalistic, overt conditions. We recorded eye movements from 33 participants during a naturalistic scene search task specifically designed to elicit frequent, unconstrained gaze shifts across complex real-world environments. We hypothesized that theta rhythms implicated in covert attention would also shape the timing of overt eye movements during visual exploration. Consistent with this, saccade timing showed robust rhythmicity, with inter-saccade intervals clustering around ~200ms and spectral power focused around 3-5 Hz. However, rhythmicity alone does not imply an underlying oscillatory mechanism. Prior work has shown that simple refractory models, where saccade likelihood is transiently suppressed after each movement, can reproduce similar theta-range dynamics without requiring a central oscillator. To distinguish these possibilities, we analyzed autocorrelograms to test whether saccade timing reflects only short-range dependencies (e.g., on the immediately preceding saccade) or exhibits longer-range

temporal structure, as would be expected from an oscillatory process. While most autocorrelograms initially showed a single dominant peak, consistent with self-paced behavior, our large dataset (~9,000 trials) revealed substantial across-trial variability. Thus, pooling over heterogeneous trials or applying long analysis windows, might obscure latent rhythmic structure. Preliminary analyses show that variability in saccade rate and spectral peak frequency mask periodicity at the subject level, whereas binning trials based on these features uncovers autocorrelograms with multiple peaks evenly spaced, indicative of higher-order dependencies. We will further explore how low- and high-level scene features modulate these dynamics and compare our results to simulations from both refractory and oscillatory models to determine which best accounts for the temporal structure of exploratory saccades. Our results are expected to advance understanding of the nature of saccade timing, which has important implications for interpreting neural rhythms in perception and attention.

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Poster

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Program #/Poster #: PSTR087.09/CC15

Topic: I.01. Attention

Title: Selective Attention and Perceptual Load Modulate the N170: Evidence from Face-Number Priming Tasks.

Authors: N. BRUNET, M. KRDIKASHYAN, *D. LIERNUR;
California State University, San Bernardino, San Bernardino, CA

Abstract: The N170 event-related potential (ERP) is a well-established neural marker of early face processing, typically associated with activity in the fusiform face area. However, its sensitivity to top-down attentional modulation and perceptual load remains underexplored. We conducted two EEG studies (N=24 and N=21, respectively) to investigate how selective attention to facial versus non-facial features modulates the N170 under varying cognitive demands. In Study 1, participants viewed randomized images of emotionally expressive faces (happy or fearful), each superimposed with a small black number (even/odd) in a white circle. Before each image, one of four questions directed attention either to the facial expression or to the number. Surprisingly, N170 amplitudes were significantly greater when attention was directed to the number rather than the face, suggesting a possible attentional suppression or perceptual gating effect under low cognitive load. Study 2 introduced increased cognitive complexity: images now contained two colored numbers (blue/red, even/odd) either on or off the face, and a broader range of prompts directed attention to number identity, number location, or eye color (brown vs. non-brown). Contrary to Study 1, N170 amplitudes were now significantly larger when attention was directed to facial expression, supporting the idea that under higher perceptual load, the

neural system prioritizes holistic face processing. Notably, attention to eye color elicited the smallest N170, possibly due to localized rather than configural processing demands. Furthermore, no significant N170 differences emerged between number locations (on-face vs. off-face), indicating that spatial location of non-facial attention may not override processing biases when perceptual load is high. These findings highlight that the N170 is not only sensitive to face perception per se but also modulated by attention modality and task load, consistent with load theory and models of holistic versus feature-based visual processing. This work refines our understanding of how early visual processing stages are dynamically influenced by cognitive context.

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Poster

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Title: Modulation of human single neurons during selective attention and change detection to what and when dimensions of auditory sequences

Authors: *Y. A. AYALA[1], R. CUSINATO[2], R. M. CALMUS[1], J. I. BERGER[1], H. KAWASAKI[1], M. A. HOWARD, III[1], A. TZOVARA[2], C. I. PETKOV[1];
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Abstract: The ability to attend to changes in events (*'what'*) and *'when'* they might occur is fundamental for guiding our behavior in spectro-temporally dynamic environments. Here, we aim to determine how responding to selectively attended changes in either the identity of sounds or their expected occurrence in time influences neurons recorded in neurosurgical patients with refractory epilepsy. We report intracranial recordings from eight patients collected with FDA-approved AdTech Stereo EEG electrodes, which contains microwires at the tip capable of recording single unit activity. Patients listened to auditory sequences that consisted of two nonsense words (0.4 s in duration) presented with either short (1 s) or long (2.5 s) stimulus onset asynchronies (*i.e.*, delays). Both stimulus and delay change were independently manipulated to occur with low (0.15) probability. Across blocks, the patients were instructed to press a key when they detected a change in either the identity of the stimuli (*'what'*) or the delay (*'when'*) and to ignore the other dimension. In separate blocks the participants either listened passively to the sound sequences or pressed a button each time a sound was presented. Participants showed

higher performance in the control (0.91 ± 0.05) and the *'what'* change detection task (0.94 ± 0.03) compared to the *'when'* condition (0.60 ± 0.08). Similarly, response times were faster in both the control (0.37 ± 0.10 s) and *'what'* detection tasks (0.69 ± 0.11 s) than in the *'when'* condition (1.30 ± 0.31 s). Neuronal activity was recorded in several brain structures, including insula (n = 126), hippocampus (n = 95), para-hippocampal gyrus (PHG; n = 58), amygdala (n = 103), basal ganglia (n = 33), cingulate (n = 90) and thalamus (n = 36). A larger proportion of neurons across areas, except in the PHG, changed their firing rate during the button pressing response (19-69%), with a smaller proportion responding during the sound presentation (6-42%). For neurons in most areas, the *'what'* and *'when'* changes elicited larger spiking activity for the respective attended dimension during the active detection than during the passive listening condition. Interestingly, in the hippocampus, we observed neurons with a pronounced decrease in firing rate when participants attended to and responded to the event changes. The results provide first indication of how human neurons across brain areas are modulated when patients allocate attention in order to detect changes in either the content or the expected time of occurrence of acoustic events.

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Poster

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Topic: I.01. Attention

Support: DOD Award RH220059

Title: Representational similarity analysis of EEG reveals multiple spatiotemporal dynamics of auditory selective attention

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Abstract: Auditory selective attention requires coordination of multiple neural processes that operate at different timescales, each implemented by different neural architectures. Listeners can achieve the same outcome by attending to a spatial location or a specific talker, but the underlying mechanisms remain partially understood. We applied representational similarity analysis (RSA) to EEG data collected during auditory attention to examine time courses and topographies of these different attention processes. Each trial began with a visual cue of attention type (spatial, talker, or no-attention), followed by an auditory cue specifying the exact target (spatial: location; talker: voice). Four overlapping syllables (/ba/, /da/, or /ga/) then played, one of which matched the cued target, and participants reported its identity. 21 conditions included

spatial attention (left, right), talker attention (male, female), and passive listening. EEG power spectra were computed and averaged within five frequency bands (delta: 2-4 Hz, theta: 4-8 Hz, alpha: 8-14 Hz, beta: 14-20 Hz, gamma: 20-50 Hz) to assess oscillatory activity. Two epochs were extracted from each trial: “cue” for preparatory attention, and “stimulus” for attention during sensory input. For each frequency band and the time-domain EEG signal, a cross-validated linear support vector machine (SVM) trained at each subject and time point classified between each pair of conditions. SVM accuracies yielded a time series of representational dissimilarity matrices for each frequency band and the time-domain signal. The channel-space weighting of each classifier allowed us to probe the spatial structure of neural information. We observed clear effects of attention vs. passive listening in all frequency bands in both the cue and stimulus epochs. In the time-domain signal, cue effects were transient (possibly sensory-driven responses), while oscillatory effects arose gradually and persisted across the trial. The alpha band showed the strongest effect during stimulus presentation, with time-domain signal, theta, beta, and gamma showing moderate differences. Topographies of the most informative channels show posterior contributions of alpha power, with two distinct time dynamics: one reflecting trial-long attention and another that faded after the target onset. The information encoded by alpha power and the time-domain signal appears largely independent of one another, suggesting that this approach captures multiple neural mechanisms. This work shows that RSA is a powerful analytical approach for revealing the dynamics of endogenous attention, advancing our understanding of cognitive control.

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Poster

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Title: Whole Head High Density fNIRS for Complex Scene Analysis

Authors: *S. DUWADI[1], D. ROGERS[2], A. BOYD[1], L. CARLTON[1], Y. ZHANG[1], A. KAWAI GAONA[1], B. ZIMMERMANN[1], W. OBRIEN[1], A. VON LÜHMANN[3], D. A. BOAS[1], M. A. YUCEL[1], K. SEN[1];
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Abstract: Complex Scene Analysis (CSA) enables the brain to focus on a single auditory or visual object in crowded environments. While this occurs effortlessly in a healthy brain, many hearing-impaired individuals and those with neurodivergent conditions such as ADHD and

autism experience difficulty in CSA, impacting speech intelligibility (Dunlop et al., 2016). We propose using high-density (HD) functional Near-Infrared Spectroscopy (fNIRS) for whole-head brain imaging during complex scene analysis (CSA) in naturalistic settings. This approach allows analysis of cortical activity patterns, with potential applications in enhancing brain-computer interface technologies.
Our experimental design mimics an ecologically valid cocktail party scenario in both overt and covert contexts. In the overt scenario, 3-second audiovisual movie clips are presented simultaneously at 30 degrees to the left and right. Prior to each clip, a 2-second spatialized white noise cue is paired with a white crosshair on the corresponding screen, guiding subjects on which direction to focus, with eye movements allowed. In the covert scenario, subjects are exposed solely to spatialized audio from the same set of movies. Here, the 2-second spatialized white noise serves as the cue, directing their attention, while they maintain a gaze on a central screen displaying a static white crosshair. For eye control tasks, the subjects move their eyes 30 degrees to the left and right. fNIRS data were collected from 15 subjects with a whole head, high density cap layout. Our results reveal brain areas that show evoked responses in both overt and covert conditions.

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Poster

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Program #/Poster #: PSTR087.13/DD4

Topic: I.01. Attention

Title: Inter-areal phase differences in alpha oscillations are modulated by attention: an analysis of open access EEG data

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Abstract: It is well known that directing attention to a specific location improves reaction time and accuracy in response to stimuli. Recent research on neural oscillations shows that attentional function is related not only to the activity of individual brain areas, but also to neural communication between them. A hypothesis has been proposed that inter-areal communication is facilitated by modulation of phase differences in alpha oscillations between cortical areas when attention is directed (Quax et al., 2017). However, the neural mechanisms underlying this phenomenon remain poorly understood. To investigate the relationship between attention and inter-areal phase differences in alpha oscillations, we analyzed two open-access electroencephalography (EEG) datasets recorded during visual attention tasks. We focused on the occipital and parietal channels, where alpha power is modulated by visual attention. After

applying a band-pass filter (7-13 Hz) to the EEG signals, the instantaneous phase difference was calculated using the Hilbert transform for 12 channel pairs (P4-PO4, P4-O2, PO4-O2, P8-PO8, P8-O2, PO8-O2, P3-PO3, P3-O1, PO3-O1, P7-PO7, P7-O1, and PO7-O1). We then computed the intertrial average of the phase difference obtained for each channel pair. A two-tailed Wilcoxon signed-rank test was performed at each time point to compare the phase differences between the experimental conditions where attention was either directed or not directed. For subsequent analysis, we selected the time window in which attentional modulation of alpha power was most pronounced in each dataset. In the first dataset (van Ede et al., 2019), eight out of 12 pairs showed time points with statistically significant differences between 500 and 800 ms after cue onset ($n=25$, $p<0.05$). In the second dataset (Schneider et al., 2020), on the other hand, seven out of 12 pairs showed time points with significant differences between 100 and 400 ms after cue onset ($n=14$, $p<0.05$). These findings suggest that inter-areal phase differences in alpha oscillations are modulated in accordance with attentional direction.

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Poster

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Topic: I.01. Attention

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Sloan Fellowship

Title: Effects of fine-scale attention shifts within the foveola on attention-related ERP components

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Abstract: Previous work demonstrated that covert attention can be selectively shifted within the 1° foveola, enhancing fine spatial vision. While the N2pc and SPCN are established EEG markers of attention, their presence during fine-grained foveal attention shifts, especially with stimuli close to visual resolution and spatially contiguous, remains unexplored. To investigate this, we used high-precision eye-tracking and gaze-contingent display control to maintain fine stimuli, presented just arcminutes from the center of gaze, at a fixed retinal location amid constant fixational eye movements.Twenty-two participants attended to a cued color (red or green, counterbalanced across blocks). While fixating, two 0.12° squares (red and green) were presented 0.33° left and right of fixation. One square contained a small gap at either the top or bottom, and participants reported the gap location as quickly and accurately as possible. In informative blocks, the gap always appeared in the cued stimulus; in uninformative blocks, its

location was random. We tested two spatial configurations between the target and distractor: separated (0.42° edge-to-edge) and contiguous (0°). High-precision eye-tracking and retinal stabilization ensured that stimuli's lateralization was maintained throughout the trial.Participants showed improved accuracy and faster responses on informative versus uninformative trials. In addition, the attentional gain for response accuracy increased with target-distractor separation. The N2pc was observed in both conditions; it showed a larger amplitude for informative trials and was unaffected by target-distractor separation. In contrast, the SPCN was exclusively present when the target and distractor were spatially separated, regardless of cue informativeness. These findings demonstrate that fine-scale attentional shifts within the foveola reliably elicit attention-related ERP components. Notably, while the SPCN is modulated by the spatial separation between target and distractor, the N2pc is modulated only by cue informativeness.

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Poster

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Topic: I.01. Attention

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Title: Multivariate Decoding of Visibility and Attention Reveals Distinct Oscillatory Signatures of Perceptual Selection

Authors: *I. AKDOGAN[1], H. OGMEN[2], H. KAFALIGONUL[3,1];
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Abstract: Perceptual awareness is not merely a passive reflection of external stimuli—it is actively constructed through neural processes that prioritize, suppress, and reshape sensory input. Two key contributors are *selective attention*, which enhances the processing of behaviorally relevant information while suppressing irrelevant information, and *visual masking*, which suppresses target visibility. The relative contributions of oscillatory activities to these processes—and how they jointly shape encoding—remain rarely examined together. We used multivariate EEG decoding to assess how frequency-band specific phase and power dynamics differentially represent attentional load and masking. We analyzed EEG data (n=20) from a metacontrast masking paradigm with varying attentional load (low vs. high) (Catak et al., 2024). In this paradigm, increased load led to greater masking, particularly at long SOAs. To decode neural representations, we trained multivariate support vector machine classifiers separately on

low-frequency (<20 Hz) power and phase features. Classification was performed across SOA, masking, and attentional-load conditions, allowing us to assess the spectral and temporal profiles of visibility- and attention-related neural signals. Both masking and attentional load were significantly decodable from EEG activities over occipital and parieto-occipital electrodes ($p <$ .01), confirming that information about perceived target visibility and attentional load is embedded in distributed low-frequency oscillations. Across all conditions, phase-based classifiers consistently outperformed power-based classifiers ($p <$ .01), indicating that phase dynamics carry more discriminative information for these processes than oscillatory amplitude alone. Spectral patterns revealed that delta and theta activities contributed most to decoding visibility suppression, while theta and alpha bands were more informative for classifying attentional load ($p <$ .01). Further cross-condition classification was applied to test for representational similarity between attention and masking, assessing whether their overlapping spatiotemporal profiles reflect convergent encoding or merely coincident activity. The results demonstrated limited generalization between neural representations of attentional load and mask-induced suppression on perceived visibility, indicating that these processes engage distinct neural codes in the low-frequency domain—a novel dissociation revealed within a unified decoding framework (Supported by the Scientific and Technological Research Council of Turkiye, BIDEB 2211, ARDEB 119K368).

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Poster

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Program #/Poster #: PSTR087.16/DD7

Topic: I.01. Attention

Support: NINDS Grant R01NS021135
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Title: Visual search: a false dichotomy

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Abstract: We continuously navigate the visual environment using attention to allocate our limited cognitive resources toward a subset of relevant items. Prominent models of attentional control describe a dichotomy between top-down (goal-driven) and bottom-up (stimulus-driven) mechanisms (Treisman & Gelade, 1980; Wolfe, 2014, 2018). Top-down refers to internal guidance based on prior knowledge, while bottom-up is driven by external salience. Macaque

studies have shown that top-down and bottom-up processes involve frontal and parietal regions, respectively, with increased frontoparietal synchrony in the beta (13-30 Hz) and gamma (>30 Hz) bands around eye movements (Buschman & Miller, 2007). These studies suggest that top-down control originates in the frontal cortex, whereas bottom-up control involves the parietal cortex. However, this dichotomy has been questioned, as both processes appear to rely on a shared frontoparietal network (Awh et al., 2012; Katsuki & Constantinidis, 2012).
We investigated the dynamics of this network to determine whether attentional systems are distinct or share a neural infrastructure. We analyzed intracranial EEG (iEEG) from 24 patients with epilepsy (1,399 electrodes post-preprocessing) during a visual search task adapted from Li et al. (2010). Participants identified a target among four triangle stimuli defined by color and orientation. In the Search condition, the target matched distractors in color; in Popout, it differed. We performed time-resolved power spectral analysis, single-trial high-frequency activity (HFA; 70-150 Hz) estimation, and inter-areal coherence measurements. Our analyses confirmed condition-specific coherence patterns: higher ~25 Hz coherence for Search and ~35 Hz for Popout near response onset. However, time-varying analysis showed similar neural dynamics in both conditions. HFA propagated from occipital to frontal and parietal cortices, then to motor/somatosensory areas, with more sustained activation in Search, partially due to reaction time differences.
Classification and permutation testing showed Search and Popout could be distinguished by alpha/beta power, particularly after 500 ms and around response onset. Yet, both conditions exhibited similar stimulus-locked and response-locked dynamics: low-frequency (alpha/beta) power decreased and high-frequency power increased in frontal and parietal regions. These findings suggest that top-down and bottom-up attention are not strictly separable, instead they are better conceptualized as intricately intertwined, drawing on a common neural architecture.

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Poster

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Topic: I.01. Attention

Title: Attentional recovery in unilateral spatial neglect: a longitudinal electroencephalography case study using the posner task

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Abstract: Unilateral Spatial Neglect (USN), often following right hemisphere stroke, impairs attention to the left space. Although the neural mechanisms of USN have been examined in cross-sectional studies, longitudinal changes during recovery remain unclear. We therefore

conducted a longitudinal electroencephalography (EEG) and neuropsychological study in a single USN patient. The patient, a 44-year-old man, developed USN after a right putaminal hemorrhage. Clinical assessments were performed at 70 days (Phase 1), 151 days (Phase 2), and 200 days (Phase 3) after symptom onset. Behavioral Inattention Test (BIT) scores improved from 136 to 146 between Phases 1 and 2. We combined event-related potentials (ERP) from a Posner cueing task with MRI-based white matter tractography to comprehensively assess functional, structural, and behavioral changes. The Posner task can assess voluntary attention shifts and reorientation. In this study, it included 152 trials (valid, invalid, neutral, and catch), and reaction times (RTs) were recorded via key press. EEG was recorded with a 32-channel eego sports system (ANT Neuro), and the patient's ERP data were compared with healthy controls (n=15). Across the three phases, RTs were shortest in Phase 2, with a significant difference between Phases 1 and 2 ($p<0.001$, ANOVA). RTs were consistently slower for left targets, with significant side differences in invalid ($p=0.007$) and neutral ($p=0.048$) trials. Left target omissions occurred only in Phase 1. MRI revealed lesions involving the middle longitudinal and inferior fronto-occipital fasciculi. In the ERP analysis, we focused on anterior directing attention negativity (ADAN), which normally appears contralateral to the direction of attention. In Phase 1, ADAN appeared in the left hemisphere for both cue directions, indicating compensatory activation of the intact hemisphere and a persistent attentional bias toward the right. In Phase 2, ADAN shifted to the right hemisphere regardless of cue direction, possibly reflecting partial recovery of attention to the left. In Phase 3, ADAN appeared contralateral to the cue direction, resembling controls, suggesting a reestablished interhemispheric balance. These observations suggest a nonlinear recovery trajectory: early right-hemisphere dysfunction with compensatory left-hemisphere activation, followed by transient right-hemisphere hyperactivation and eventual bilateral reorganization. Transient hyperactivation may represent an adaptive stage amenable to attention training or non-invasive stimulation. The study was approved by the Kio University Institutional Review Board; written informed consent was obtained.

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Poster

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Topic: I.01. Attention

Support: Wellcome Trust Institutional Strategic Support Fund at the University of St Andrews, grant code 204821/Z/16/Z (DB)

Title: Disease mechanisms in spatial neglect: displaced locus of attention in response to salient objects

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Abstract: *Background* Spatial neglect (SN) is a common and disabling outcome after a unilateral (typically right) brain hemisphere injury. Patients lack awareness for people and objects in the contralesional (typically left) hemifield. It has been reported that in SN salient, task-irrelevant objects (distractors) presented in the left, neglected visual hemifield can, paradoxically, speed up visual detection of right, non-neglected targets (Vossel & Fink, Cortex, 2016). This is intriguing because it contrasts with the findings in healthy elderly individuals. Notably, this observation offers the opportunity to test a new hypothesis about the causal mechanism in SN. If, in left SN an incorrect attentional priority map misrepresents the location of salient objects, displacing them rightwards, then attention will not be deployed at the exact location of the distractor, but rightward of it, at a specific location (Hypothesis 1; Balslev & Odoj, Neuropsychologia, 2019). Alternatively, the distractor could act as a spatial-unspecific warning, improving perception regardless of target location (Hypothesis 2). *Methods & Results* To distinguish between alternatives, we asked participants with a right hemisphere stroke with SN (N=12, 4F) and without SN (N=11, 7F) to discriminate a target letter presented at various lateral eccentricities in their right visual hemifield. A distractor letter was flashed in the left hemifield shortly before the target in half of the trials. When averaged across all target locations, there was no statistically significant difference in the distractor effect across groups. To find out whether a paradoxical distractor effect in SN could have occurred at a specific target location only, we identified the peak positive distractor effect in each individual (PPDE, the largest difference in reaction time for target discrimination in the absence minus in the presence of a distractor across target locations). PPDE was larger in the SN vs stroke control group (independent samples t-tests, two-sided, unequal variances, p=0.03). To assess the reliability of this location within each participant, we split individual data into a training set, to identify PPDE location, and an independent, test set to extract the value of the distractor effect at that location. PPDE location was reliable in the SN group (permutation test, p=0.022; the likelihood of finding a smaller distractor effect in the test set at a random location vs. the PPDE location indicated by the training set), but not in the stroke control group (p=0.76). *Discussion* In spatial neglect, the attentional priority map displaces (rather than fails to represent) salient locations.

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Poster

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Topic: I.01. Attention

Title: Mechanisms of Selective Attention in Autism Spectrum Disorder: Behavioral and Neural Insights from EEG

Authors: *M. DARRELL[1], T. VANNEAU[1], D. COEN[1], A. SEJDIU[1], S. MOLHOLM[2]; [1]Albert Einstein Col. of Med., Bronx, NY; [2]Pediatrics, Albert Einstein Col. of Med., New York, NY

Abstract: MOTIVATION In a world rich with sensory information, selective attention allows us to prioritize certain stimuli while suppressing others. These processes are influenced by social relevance; from infancy, socially-salient stimuli—like faces—are more likely to capture attention. In Autism Spectrum Disorder (ASD), attentional atypicality is prevalent, with altered selective attention likely contributing to hallmark symptoms of autism. Previous studies of attention in ASD are often conflicting and rely on behavioral evidence. To date, there is no consensus on the neural mechanisms underlying altered attention in ASD. Furthermore, previous research has largely failed to explore how social stimuli influence attention in individuals with ASD. **METHODS** To address this gap, we conducted a systematic investigation of selective attention using non-social and social stimuli. Using high-density electroencephalography (EEG), we adapted a well-characterized visuospatial task to test how neural responses to non-social (houses) and social (faces) stimuli differ between ASD children and age-matched typically-developing (TD) counterparts (n=40, 13-17 YO, full-IQ >80). Comparison of neural markers well-known to reflect attention related enhancement (early sensory response) and suppression (increased alpha-band power) were used to quantify selective attention. A comprehensive battery of clinical and cognitive assessments was collected to assess the relationship between attentional profiles, ASD symptomatology and neural markers of attention. **RESULTS** Preliminary analyses of behavioral and neural data indicate markedly impaired selective attention in ASD. Specifically, individuals with ASD showed less attenuation of responses to unattended stimuli compared to TD. Moreover, neural markers and behavioral performance were found to correlate with clinical measures of attention, establishing a link between neurophysiological responses and attentional profiles. **CONCLUSIONS** The current data support impaired selective attention in children with ASD, with no differences observed between social and non-social stimuli. Preliminary analyses suggest this impairment reflects reduced distractor filtering, consistent with more broadly distributed attention in ASD. Ongoing analyses aim to disentangle within-group heterogeneity in attentional profiles, with the goal of informing personalized diagnostic tools and intervention strategies tailored to the distinct attentional patterns observed in ASD.

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Poster

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Topic: I.01. Attention

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Title: Multiscale entropy is related to dietary iron status in resting state EEG data

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Abstract: Dietary iron deficiency in the absence of anemia (IDNA) affects billions of people worldwide, particularly women of reproductive age. Research suggests that IDNA has a wide range of negative effects on brain functioning and cognition. Although studies employing electroencephalography (EEG) have revealed a number of negative effects of IDNA in both the time- and frequency domains, to date there have been no attempts to characterize the effects of IDNA on the temporal dynamics of whole brain interactions. To address this issue, we applied multiscale entropy (MSE) analysis to resting-state EEG data collected from IDNA (n = 21) and iron sufficient (IS, n = 21) women. MSE analysis provides unique information on the complexity of brain-wide neural dynamics over a range of timescales, with higher complexity being associated with higher resilience with respect to the effects of disease states, aging, etc.. For example, these measures have been used to show that high functioning older individuals have more complexity in signals collected at frontal, parietal, and temporal electrodes than do low functioning individuals (Iinuma et al., 2022). We thus sought to determine whether IDNA as associated with lower complexity in otherwise healthy college-aged women. We used unpublished data from a previous study (Newbolds & Wenger, 2024) in which two groups of age- and ethnicity-matched women, one IDNA and one IS, sat for five minutes in an alert resting state while concurrent EEG was recorded. Following standard preprocessing (Delorme, 2023), an MSE analysis (Costa et al., 2005) on this data revealed that entropy was higher overall for the IS than the IDNA group, with significant differences appearing primarily at longer time scales and under right frontal and left and right parietal electrodes. These results suggest that IDNA may negatively affect long-distance interactions among brain regions and that this could conceivably be a source of diminished cognitive function in IDNA. These results are, to our knowledge, the first application of complexity measures to study the effects of IDNA on brain function in otherwise healthy women and support the hypothesis that IDNA negatively impacts whole-brain interactions and the use of higher cognitive functions.

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Topic: I.01. Attention

Title: A Systematic Meta-Analysis of Electrode Coverage Strategies in Deep Learning and Machine Learning-Based EEG Classification of ADHD

Authors: ***T. FU**[1,2], C.-H. LEE[5,6], J.-E. DING[7], Y. HUANG[9,10,3,4], F. LIU[8];
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Abstract: Although deep learning (DL) and machine learning (ML) models have demonstrated considerable promise in classifying attention-deficit/hyperactivity disorder (ADHD) using electroencephalogram (EEG) signals, most existing studies overlook the impact of spatial electrode placement on model performance, particularly regarding the selection of scalp regions. While many studies focus on biomarkers within specific frequency bands such as delta and theta, it remains unclear whether features extracted solely from frontal regions—which are commonly implicated in ADHD pathophysiology—are sufficient for high-accuracy classification or whether incorporating full-scalp data provides meaningful performance gains. In this work, we conducted a systematic meta-analysis reviewing studies from PubMed, Embase, and Springer that applied DL and ML to EEG-based ADHD classification in pediatric populations. This survey was stratified according to electrode coverage: (1) frontal-only channels (e.g., Fz, F3, F4, F7, F8) and (2) full-scalp arrays (more than 10 channels across multiple brain regions). To assess the performance of our meta-analysis, evaluation metrics including accuracy, area under the curve (AUC), sensitivity, and specificity were extracted and compared across groups. Existing findings demonstrate that frontal frequency band features can achieve classification accuracy comparable to full-scalp models. However, current studies exhibit considerable variability in preprocessing methodologies and model architectures, limiting direct comparisons. Furthermore, few studies have systematically evaluated the relative contributions of different scalp regions or utilized artificial intelligence (AI) techniques to reveal underlying spatial-frequency relationships. These limitations represent significant gaps in the current deep learning and maching learning-based ADHD research landscape. Our investigation represents the meta-analysis can provide effective insights for diagnostically selecting scalp regions in EEG-based ADHD classification using DL and ML models. By comparing frontal-only and full-scalp input strategies and integrating insights from AI models prediction, our study provides a new direction on whether a regionally focused approach can achieve comparable performance while reducing data complexity and acquisition burden. These findings will inform both clinical EEG protocols and future AI model designs for the diagnosis of ADHD.

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Topic: I.01. Attention

Title: Tipping the Balance: ADHD Traits Shift Attention Toward Bottom-Up Capture in an Inattentional Blindness Task

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[1]Ctr. for Data Sci., New York Univ., Brooklyn, NY; [2]Psychology, New York Univ., New York, NY; [3]Data Sci., New York Univ., New York, Afghanistan

Abstract: Whether attentional deficits constitute a core feature of Attention-Deficit/ Hyperactivity Disorder (ADHD) is still contested. Recent work on inattentional blindness suggests that the balance between top-down (endogenous) and bottom-up (exogenous) control can be indexed by the threshold at which an unexpected moving object (UMO) captures attention. We asked whether this threshold differs in adults with elevated ADHD symptoms. Specifically, research participants (n > 200) completed the Adult ADHD Self-Report Scale (ASRS-31) and a two-trial selective-attention task. On each trial black and white dots moved randomly while participants counted midline crossings of one color (endogenous task). During the second trial a triangular UMO traversed the display at one of six log-spaced speeds. Object detection and counting accuracy were recorded. We used logistic regression to estimate the threshold at which endogenous attention is overridden, using depression (as measured by the PHQ-9) and anxiety (as measured by the GAD-2) as covariates. Replicating our earlier work, UMO detection increased monotonically with speed. Participants exceeding the ASRS screening cut-off showed a left-shifted detection function compared with ADHD - peers while slopes were identical. Counting accuracy did not differ between groups. PHQ-9 and GAD-2 scores were highly correlated with each other, but did not predict detection performance after controlling for speed. We conclude that people with elevated ADHD symptomatology redeploy attention at lower exogenous salience, consistent with a weakened endogenous set or heightened bottom-up drive. This shift provides a quantitatively defined cognitive marker that may aid objective assessment of ADHD and clarifies why modern, distraction-rich environments prove particularly challenging for affected individuals.

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Topic: I.01. Attention

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Title: Decreased influence of distracting singleton stimuli at task-irrelevant locations

Authors: *S. E. PURNELL, D. D. THAYER, T. C. SPRAGUE;
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Abstract: During visual search, it is necessary to prioritize task-relevant stimulus features and locations and minimize negative impacts of task-irrelevant distractors. For instance, if the position of a target is known in advance, attention can be restricted to a limited set of locations, enhancing relevant locations while minimizing the influence of distractors at irrelevant locations (Kastner et al., 1999). Critically, spatial attention may differentially affect interference from color- and motion-defined distractors (Leonard et al., 2015; Liu & Mance, 2011). While the general phenomenon of task-dependent attentional capture is well-established, it remains unknown how these effects generalize across different visual features (e.g., color, motion, shape) defining task-relevant and salient stimuli. Here, we had participants perform a visual search task where they covertly searched for a unique target item defined by a specific feature dimension (unique color, unique motion, unique shape) in an 8 item array and reported the orientation of a line (horizontal or vertical) superimposed on the target. On most trials, participants were centrally cued to attend to half of the search array (top, left, bottom, or right; 4 of 8 items), in which the target would always appear, limiting the number of relevant search locations. Half of all trials contained a task-irrelevant, singleton distractor within a non-target dimension (e.g., color target and motion distractor), defined as the opposite color, motion direction, or shape (Li et al., 2019) of all other items in the array along a 360-degree feature space. When a distractor was present, it could either be within or outside of the cued hemifield. The difference in reaction time between the distractor-present and distractor-absent trials indicated that while a distractor always slowed response times, restricting relevant locations, via cueing, mitigated distractor-induced capture when the distractor was outside (vs within) the cued hemifield. These results indicate that top-down goals may influence visual selection and minimize distractor processing to guide visual search, and support future studies into how the neural mechanisms governing the interaction between task relevance and stimulus salience during covert visual search may sculpt neural representations in feature-selective regions of the cortex.

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Poster

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Program #/Poster #: PSTR087.24/DD15

Topic: I.01. Attention

Title: The Dynamics of Engaging Attention during Rapid Serial Visual Presentation

Authors: *L. R. QUINN[1], A. R. KIMATA[2], T. WATANABE[3], W. F. ASAAD[4];
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Abstract: Accurate identification of task-relevant stimuli from the constant stream of visual inputs is critical for guiding appropriate behavioral responses. Prior work has produced conflicting theories about the factors constraining the deployment of attention. Specifically, the rapid allocation of attention to a target may be capacity-limited or limited by the dynamics of visual and attentional processing. Here, we apply a modified version of a single-stream rapid serial visual presentation (RSVP) paradigm that incorporates non-informative "filler" stimuli to disentangle dynamic vs. resource-limited mechanisms. We found that short presentation intervals were compatible with high performance when the interval between the cue and target was sufficiently long. This effect was largely independent of the presence, type, or history of fillers, consistent with the notion that RSVP performance is limited primarily by the temporal dynamics of activating or allocating attention.

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Title: Ethologically based attention and its interdependence with goal-driven attention

Authors: J. ZHU[1], ***T. P. HAMEL**[1], J. FORD[1], A. BURMAN[1], D. J. SPERKA[1], X. CHEN[2,1]; [1]UC Davis Ctr. for Neurosci., Davis, CA; [2]Dept. of Neurobiology, Physiology, and Behavior, Univ. of California, Davis, Davis, CA

Abstract: Ethologically based attention (EBA) prioritizes stimuli of inherent behavioral significance shaped by evolutionary pressures and experience, such as faces signaling social relevance or food indicating nutritious input. Recent studies have shown that this form of attention exerts a significant influence on attentional deployment in humans and non-human primates. However, its impact on perceptual sensitivity—an essential indicator of attentional processing—and its interaction with goal-directed attention (GDA) remain unclear. To address these questions, we conducted two behavioral experiments. In the first experiment, we assessed perceptual sensitivity changes associated with EBA using stimuli depicting faces, food, and landscape plants. Employing a change detection task, ethologically based attention was manipulated via stimulus pairs: both high-salience (HH), both low-salience (LL), or mixed (HL, LH). Participants demonstrated significantly higher perceptual sensitivity to baby faces and food

images compared to landscape plants, suggesting a graded prioritization mechanism within ethological salience processing. In the second experiment, we evaluated the interaction between EBA and GDA. In the experiment, the EBA is manipulated orthogonally to GDA. Goal-directed attention is tested using cueing blocks, where cues indicate the likely location (left/right, 90% validity) of orientation changes. On combined-attention trials, high-salience images were either congruent or incongruent with the cue location. Our findings reveal a clear interdependence between EBA and GDA. Specifically, EBA robustly modulates perceptual sensitivity when stimuli are unattended, whereas this modulation diminishes under conditions of directed attention. Collectively, our preliminary findings show a clear graded effect of EBA on visual sensitivity and illuminate its interdependence with goal-directed attention. These results provide a clear behavioral foundation for future neurophysiological studies in non-human primates.

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Poster

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Topic: I.01. Attention

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Title: Implementing the speed-accuracy tradeoff online: a replication and extension

Authors: *N. CROTTY[1], M. A. GRUBB[2];
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Abstract: Sudden peripheral onsets engender reflexive shifts of visual attention that are spatially specific and lack accompanying eye movements, known as covert exogenous spatial attention. This phenomenon increases asymptotic discriminability and hastens the rate of information accrual at attended locations. Such findings are garnered from a speed-accuracy tradeoff (SAT) protocol, which depicts changes in perceptual sensitivity relative to time allotted for visual information accumulation. However, SAT protocols require extensive data collection due to the difficulty of the associated task and their multitude of experimental conditions. Online psychophysics could mitigate this issue by providing a more efficient method for data collection. Here, we developed an online version of the SAT protocol and replicated the classical findings from in-person studies. Our online protocol began with a four-phase preliminary study. In the fourth “screening phase”, participants were presented with two peripheral Gabor patches on the left and right of fixation, and reported the orientation of the target post-cued Gabor (clockwise/ counterclockwise from vertical) after a variable delay interval (33ms-2133ms). Prior to target onset, an abrupt-onset cue flashed on the same (valid) or opposite (invalid) side of the target. Participants were invited back for two additional sessions if they met two inclusion criteria in the screening phase (accuracy > 65%, missed responses < 10%). Out of 114 participants, 16 met

both criteria and completed ≥ 1 additional session, constituting the Experiment 1 dataset. The 3-parameter SAT model was fit to the Experiment 1 data through a Bayesian generalized linear mixed effects (GLME) approach, quantifying validity-driven changes in asymptotic discriminability (*d'*) and processing time. To mirror classical in-person findings, we expected to see asymptotic discriminability increase and processing time decrease in valid relative to invalid trials. We observed a between-validity increase in discriminability, but processing time was unchanged, attributed to the task not being sufficiently difficult. We then invited all passing participants to complete a second experiment, with several methodological changes designed to increase task difficulty. An identical SAT modeling approach revealed an increase in discriminability and decrease in processing time between valid and invalid trials. Furthermore, asymptotic performance was lowered in Experiment 2, indicating a more difficult task. Thus, we successfully implemented the SAT protocol online, providing a framework for future web-based studies of covert exogenous spatial attention.

Disclosures: N. Crotty: None. **M.A. Grubb:** None.

Poster

PSTR088: Prefrontal Cortex

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR088.01/EE2

Topic: I.03. Decision Making

Support: This work was supported by a grant from the Simons Foundation- NC-GB-CULM-00003265-03

Title: MRI-guided electrophysiology of brain-wide inputs to mouse prelimbic cortex

Authors: *Y. BROWNING[1], G. F. LYNCH[1], S. N. BURCKHARDT[1], M. T. SUMMERS[1], D. LEE[2], S. TOTTEN[3], K. SVOBODA[1], J. H. SIEGLE[1];
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Abstract: Every neocortical area receives diverse inputs from across the brain, yet how these inputs shape local activity remains unclear, due to the technical challenge of measuring spikes across anatomically connected brain-wide circuits. Medial prefrontal cortex (mPFC) is necessary for tasks in which animals integrate action and reward history to guide future choices. In one such task, neurons in prelimbic cortex (PL), a subregion of mPFC, exhibit slow changes in baseline firing-rate that encode action value. However, the origin of these long-timescale dynamics remains uncertain: they could be generated within PL itself, be inherited from an input, or arise from recurrent connections between brain regions.
Distinguishing these alternatives requires simultaneous recordings from PL and its major input regions. Many of these regions are small (<500 μm in diameter) and distributed throughout the brain. The field-standard stereotaxic electrode targeting, guided by skull landmarks, results in a

median error of 430 μm (IBL et al., 2025, see also Narayanan et al., 2022)—insufficient for reliably measuring activity across this brain-wide circuit.
To overcome these limitations, we produced an MRI-compatible headpost with MRI-visible fiducials. Following headpost implantation, we acquired 100 μm isotropic T1-weighted MRI volumes using manganese-enhanced imaging (Massaad et al., 2010) which we registered to the mouse Common Coordinate Framework (CCF). Targeting accuracy was validated by recording in a retinotopically-defined region of lateral geniculate nucleus (LGN), and by driving electrodes through anatomical landmarks visible in both MRI and ex vivo cleared brains. Our method improves targeting accuracy over conventional stereotaxic techniques by a factor of 2.6, achieving 166 μm precision.
We applied this method to simultaneously record neural activity in PL and many of its major input regions. By combining data across retrograde tracing experiments using rAAV or rabies vectors with the existing literature, we identified six candidate input regions with locally dense projections to PL—retrosplenial cortex, insular cortex, ventral hippocampus, basolateral amygdala, and the mediodorsal (MD) and ventromedial (VM) thalamic nuclei. We aimed to simultaneously record from all of these structures as mice performed a history-dependent two-armed bandit task. By aligning retrograde tracing results first to the CCF and subsequently to each mouse's MRI, we accurately targeted projections to specific PL recording sites. Using this approach, we report the first simultaneous electrophysiological recordings from PL and its major inputs during behavior.

Disclosures: Y. Browning: None. **G.F. Lynch:** None. **S.N. Burckhardt:** None. **M.T. Summers:** None. **D. Lee:** None. **S. Totten:** None. **K. Svoboda:** None. **J.H. Siegle:** None.

Poster

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Topic: I.03. Decision Making

Support: R01DA031695

Title: Neural signals in Retrosplenial Cortex (RSC) reflect conflict and errors during the STOP-change task

Authors: *A. G. SPERA[1], S. ASHTON[1], B. OSUALA[2], A. GUPTA[2], M. R. ROESCH[2]; [1]Psychology, [2]Univ. of Maryland, Col. Park, College Park, MD

Abstract: The retrosplenial cortex (RSC) is thought to be critical for spatial navigation, memory, and contextual learning, however, its role in cognitive control remains unclear. In particular, it is unknown how or if RSC contributes to the resolution of response conflict as defined by the competition between two competing events. The classic example of response conflict comes from the Stroop task, where participants must say the ink color of a word while ignoring the

written word. Animals may resolve conflict by attenuating irrelevant sensory information (e.g., processing the written word) inhibiting inaccurate motor output (e.g., saying "yellow"), or both. Our work on this topic focused on brain areas in the front of the brain that are associated with the motor system. We have demonstrated that conflicted signals are resolved prior to the motor response, and these directional signals are multiplexed with signals related to conflict monitoring and adaptation. From these studies, it appears that rats resolve conflict on the motor side. Yet, we have never recorded from brain areas that are arguably less motor-related and more sensory-related. Here, we focus on RSC, which is thought to contribute to both basic spatial functions and complex higher-order processes. Our goal was to determine (1) if competing spatial signals are resolved before the motor response and (2) if RSC neurons carry other cognitive control-related signals.

We recorded from single neurons in the RSC of rats performing a STOP-change task, which captures the ability to resolve spatial conflict. During task performance, in 80% of trials, a spatial cue light signaled the direction that the rat had to respond to receive reward (left or right). In 20% of trials, after the presentation of this cue, a 2nd spatial cue appeared, requiring the rats to STOP and redirect. We found that RSC neurons were directionally selective in that they fired more strongly for one spatial cue over the other on GO trials. Firing on STOP trials was indiscriminately high, even on trials where the animal was correct, suggesting that resolution of conflicted spatial signals in RSC is not necessary for accurate performance.

RSC also carried signals that were arguably more cognitive. First, average firing was higher on STOP trials and correlated with the ability to resolve conflict. Also, firing during STOP cues was significantly lower when rats failed to inhibit behavior. Furthermore, we found that activity in RSC was significantly higher after errant STOP trials. Thus, even though RSC may not contribute to the resolution of spatial signals, it may play a role in signaling conflict and detecting errors so that downstream motor conflict can be resolved.

Disclosures: A.G. Spera: None. **S. Ashton:** None. **B. Osuala:** None. **A. Gupta:** None. **M.R. Roesch:** None.

Poster

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Topic: I.03. Decision Making

Support: NIH Grant R01DA031695 (MRR)

Title: Get over it! Neural correlates of perseverance in the face of setbacks in ACC in rats performing a novel multistep task

Authors: *S. ASHTON[1], A. G. SPERA[1], A. GUPTA[2], B. OSUALA[2], M. R. ROESCH[1]; [1]Psychology, [2]Univ. of Maryland, College Park, MD

Abstract: Why is starting over harder than starting up? How do you get over failures that set you back to square one? What brain areas encode this information? To start to address these questions we have established a novel “Multi-Step Perseverance Task” (MSPT) to explore the psychological constructs that mediate how effort, perseverance and resilience influence the drive towards a goal that requires multiple steps to achieve and often results in setbacks along the way. In the task, rats respond to sequential cues, but “fall” back earlier in the sequence when they make mistakes. There are two basic trial types: on longer “3-hill” trials, rats proceed through three sequential nosepokes (either left -> center -> right or the reverse direction, pseudorandomized) indicated by cue lights, while shorter “2-hill” trials instruct the rat to skip the center poke. This allows us to explore behavior and neural firing when actions are completed before or after more (e.g., 3-hill) or less (e.g., 2-hill) work has been put into a string of actions as the rats move closer to obtaining a sucrose reward. If rats fail to maintain the 1 s poke, they “fall” varying steps to an earlier phase of the sequence, allowing for further comparison between harder (e.g., two step fall from last port back to first) and easier (e.g., one step fall from the last port to the center port) fall sequences. Preliminary data suggest that rats speed up as they move through a sequence—mirrored by an increased error rate later in the sequence—but slow down directly after errors. We are recording from single neurons in the anterior cingulate cortex (ACC) as rats perform this task. The ACC is associated with components of executive function like attention and emotion that ideally position it to guide decisions about which actions are worth the effort, combining its more classical role in error monitoring with the integration of emotional and motivational states. We will examine activity of ACC ensembles during actions that were completed before or after more or less work has been put into a sequence, as well as before or after “falls” at different phases and of different difficulties. In a relatively long task with setbacks, the rat must find a balance between the drive to keep going and an appropriate level of inhibition to prevent errors. Overall, we predict that ACC activity will reflect this balance with multiplexed signals that relate to error monitoring and value. MSPT is unique from other tasks that manipulate reward probability, magnitude or effort independent of the rat’s performance, providing novel insight into the ability to keep going and try again after mistakes, which has broad relevance to psychiatric disorders like addiction.

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Poster

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Program #/Poster #: PSTR088.04/EE5

Topic: I.04. Executive Functions

Support: R00MH117271

Title: Local signal control by prefrontal layer 6 corticothalamic neurons

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Abstract: Prefrontal corticothalamic loops are fundamental to executive function but remain poorly characterized. In sensory cortical regions, layer 6 corticothalamic neurons (L6CT) contact local interneurons to control the response amplitude, but not neuronal representation of layers 2/3 and 5 neurons. We hypothesized that prefrontal L6CT neurons served a similar gain-modulating purpose; to control signal amplitude while maintaining representations for executive function. To test this idea, we combined two-photon calcium imaging of the prefrontal cortex with optogenetic manipulation of L6CT neurons as mice performed a head-fixed cognitive flexibility task. Optogenetic pacing of L6CT neurons was timed to trial feedback epochs and intertrial-intervals of a cognitive flexibility task that demanded selective attention to one modality (e.g. olfaction) while ignoring another (e.g. whisker-vibratory stimuli). By aligning widefield red-light illumination to interframe periods through an implanted microprism, we successfully photoactivated L6CT neurons expressing ChrimsonR+ while recording 605 prefrontal neurons expressing Syn-GCaMP6f across 6 mice. After removing putative ChrimsonR+ neurons, we found that L6CT activation excited 11% while suppressing 25% of neurons, with both populations observed in all mice. Using a generalized-linear model trained to predict task features from deconvolved calcium transients, we found that L6CT activation broadly disrupted task representation, even amongst neurons not directly photomodulated. During light-off trials, suppressed neurons more strongly represented trial-outcome (correct vs error) relative to excited neurons. In contrast, during light-on trials, the representations of most excited neurons were gain-modulated. These results indicate that prefrontal L6CT neurons serve to control local cortical computations by selectively amplifying the task representation of a key group of neurons while broadly disrupting higher-order task representation amongst non-activated neurons.

Disclosures: J.J. Stout: None. **T. Spellman:** None.

Poster

PSTR088: Prefrontal Cortex

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Program #/Poster #: PSTR088.05/EE6

Topic: I.03. Decision Making

Title: Neural mechanism of the Internally driven strategy shift in the prefrontal cortex

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Abstract: Cognitive flexibility is a crucial executive function that enables animals to adapt to a constantly changing environment, involving the ability to switch between tasks and toggle

between multiple strategies. We note that a key limitation in current paradigms of cognitive flexibility research is the difficulty in disentangling the mechanisms of negative feedback from strategy shift. To overcome the limitation, we developed a novel behavioral paradigm that allows subjects to shift strategies without negative feedback, building on insights from creativity and cognitive flexibility studies. In this study, internally-driven strategy shifts were compared with error-feedback-guided shifts in rats. Our findings demonstrate that the medial prefrontal cortex (mPFC) is specifically involved in internally-driven strategy shifts. By combinationally using optogenetics and electrophysiological recordings, we observed that while a subset of mPFC neurons encoded optimal strategies prior to behavioral shifts, network perturbation paradoxically accelerated behavioral switching. These results suggest that the prefrontal network serves dual roles: it not only encodes early indicators of new strategies but also actively maintains ongoing strategy stability during cognitive flexibility processes.

Disclosures: S. wang: None. **Z. Wang:** None.

Poster

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Topic: I.03. Decision Making

Support: IBS-R002-A1; M.W.J

Title: Selective engagement of prefrontal VIP neurons in reversal learning

Authors: *J. YI[1], Y. YOON[2,3], H. JEONG[2], M. JUNG[3];
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Abstract: To gain insights into neural mechanisms enabling behavioral adaptations to complex and multidimensional environmental dynamics, we examined roles of VIP neurons in mouse medial prefrontal cortex (mPFC) in probabilistic reversal learning. Behaviorally, manipulating VIP neuronal activity left probabilistic classical conditioning unaffected but severely impaired reversal learning. Physiologically, conditioned cue-associated VIP neuronal responses changed abruptly after encountering an unexpected reward. They also conveyed strong reward prediction error signals during behavioral reversal, but not before or after, unlike pyramidal neurons which consistently conveyed error signals throughout all phases. Furthermore, the signal's persistence across trials correlated with reversal learning duration. These results suggest that mPFC VIP neurons play crucial roles in rapid reversal learning, but not in gradual value updating under stable probabilistic contingencies, by monitoring salient deviations from ongoing environmental contingencies and imposing error-correction signals during behavioral adjustments. These findings shed light on the intricate cortical circuit dynamics underpinning behavioral flexibility in complex, multifaceted environments.

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Poster

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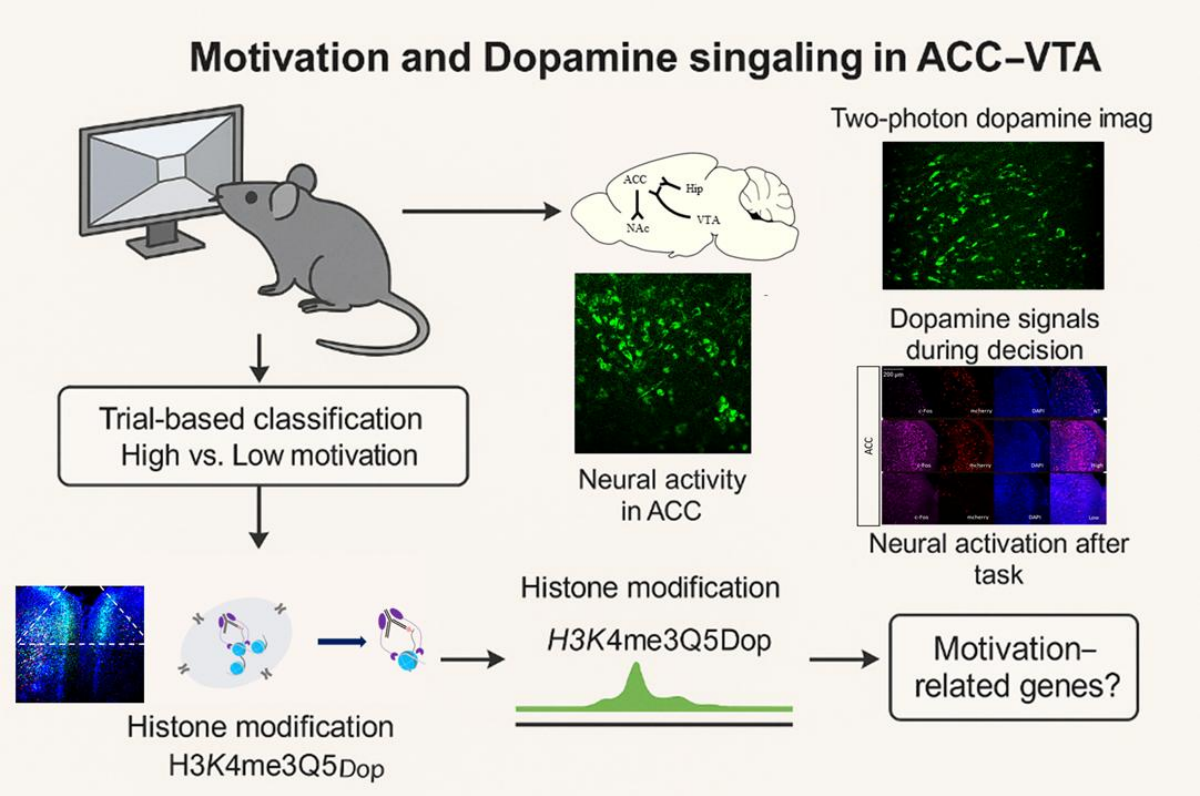
Topic: I.03. Decision Making

Support: 25-BR-05-02
RS-2024-00508718

Title: Motivational Neural Circuitry and Epigenetic Regulation in a VR-Based Decision-Making Task

Authors: *D.-S. KANG[1,2], J. KOO[1,2];
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Abstract: Motivation is essential for goal-directed behavior and adaptive decision-making, yet its underlying neural and epigenetic mechanisms remain incompletely understood. To investigate these processes, we employed a virtual reality (VR)-based T-maze task in head-fixed mice, combined with two-photon calcium imaging to capture real-time neural dynamics. Mice were stratified into high- and low-motivation groups based on behavioral performance. The high-motivation group showed significantly elevated neural activity in the anterior cingulate cortex (ACC) during correct trials, a pattern not observed in the low-motivation group. Post-task immunohistochemistry revealed increased c-Fos expression in both the ACC and ventral tegmental area (VTA), indicating task-related activation of these regions. To further dissect the circuit-level underpinnings, we performed two-photon dopamine imaging, achieving single-cell resolution of dopamine signals in the ACC during task performance. Epigenetic profiling revealed a motivation-dependent increase in histone dopaminylation (H3K4me3Q5Dop) within the ACC of high-motivation mice. CUT&RUN assays identified genomic loci enriched with this modification. These findings suggest that motivated behavior engages specific dopaminergic circuits and is associated with distinct epigenetic signatures, offering new insights into the molecular substrates of cognitive engagement.



Disclosures: D. Kang: None. **J. Koo:** None.

Poster

PSTR088: Prefrontal Cortex

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Title: Planning by Sampling Neuronal Assemblies in the Prefrontal cortex

Authors: *M. EL-GABY[1], A. HARRIS[1], M. E. WALTON[2], T. AKAM[1], T. E. BEHRENS[3]; [2]Exptl. Psychology, [1]Univ. of Oxford, Oxford, United Kingdom; [3]FMRIB Centre, Univ. Oxford, Oxford, United Kingdom

Abstract: To plan, we use prior knowledge to choose between alternative futures. The medial prefrontal cortex (mPFC) is crucial for abstract task knowledge and planning, but its underlying algorithms remain unknown. Here, we found that mPFC neuronal dynamics embed behavioural

sequences into an abstract task structure and select between them during planning. We trained mice on tasks which shared a common structure organising a sequence of goals, but differed in goal locations. Mice learned this structure, efficiently inferring new goal paths to solve new tasks. We found that mPFC neurons formed “task-structured memory buffers,” firing with a fixed lag in task-space relative to specific behavioural steps. This created assemblies that instantly encoded entire future sequences. Intriguingly, the dynamics of such assemblies are consistent with a planning algorithm. During reward consumption, the mPFC first sampled assemblies encoding past “memory” paths, but then shifted to assemblies representing future “plan” paths, which were over-sampled before execution. Our findings reveal an algorithm in the mPFC that leverages task-sculpted neural dynamics to compute goal-directed behavioural sequences and rapidly sample them to plan efficient routes.

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Poster

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Support: ERC StG MEMCIRCUIT 758032
ONE MUNICH Strategy Forum Next Generation Human-Centered Robotics

Title: Population-level reorganization during learning amid single-neuron volatility in mouse prefrontal cortex

Authors: *Y. HUANG[1,2,3], L. S. MEHRKE[1], T. W. BERNKLAU[1], L. BUSSE[2], S. N. JACOB[1]; [1]Dept. of Neurosurg., TUM Univ. Hospital, Tech. Univ. of Munich, Munich, Germany; [2]Biol. II, [3]Grad. Sch. of Systemic Neurosciences, LMU Munich, Planegg-Martinsried, Germany

Abstract: During associative learning, animals generate internal concepts of their environment and flexibly link sensory stimuli, actions, and outcomes. In rodents, the medial prefrontal cortex (mPFC) is believed to play an essential role in this process. However, how prefrontal neurons represent task-relevant variables and convert these to behavioral choices under changing task demands is not known. Here, we trained head-fixed mice (n=12) to form stimulus-action-outcome associations with implicit rule switches, requiring the animals to choose left or right according to an auditory cue's location or frequency. As the animals acquired the task, we recorded from the mPFC (prelimbic area) using chronic single-photon fluorescent calcium imaging with cellular resolution (head-mounted miniature microscopes, “miniscopes”; GCaMP6f targeted to excitatory projection neurons). 2685 neurons were recorded in more than one imaging session. 70% of neurons were task-related and showed trial-epoch specific activity. Selectivity

for cue location and frequency increased across learning and changed according to behavioral demands. Four mice completed all tasks with good imaging quality, from which we were able to record 170 neurons in 9 sessions spanning up to 3 months. The activity of these neurons was remarkably stable across sessions. Surprisingly, however, cue selectivity peaked in individual sessions, but was relatively weak in others. Further analysis revealed that the neuronal population collected information from both sensory dimensions during cue presentation and formed a subsequent choice representation, which strengthened across learning. As learning progressed, the hyperplane classifying task-relevant sensory information aligned with that separating behavioral choices. This alignment was lost, but recovered after rule switches. We developed sparse Tensor Component Analysis (sTCA), which allowed us to identify four dynamic activity patterns (components) that could be linked to specific neuronal subpopulations. These patterns separated task-relevant from irrelevant information. Across sessions, the contributions of individual neurons to these components showed dynamic rotation and inversion in state space, reflecting the switch from a location to a frequency context, as well as the reversal of the frequency rule. Our results suggest that learning and adaptive behavior are supported by the directed reorganization of prefrontal population-level activity, which develops in spite of highly dynamic activity and recruitment of individual neurons.

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Title: Neurotrophin signaling is essential for socially-guided choice

Authors: *T. T. TOWNER[1], E. SEO[1], A. LEAHY[1], E. MCCALL[1], K. XING[1], T. ANAND[1], S. L. GOURLEY[2];
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Abstract: The neurotrophin, Brain-derived neurotrophic factor (BDNF), within the medial orbitofrontal cortex (MO), is a key regulator of social behaviors. The social incentivization of future choice (SIFC) task allows us to evaluate the process by which prior social experiences

promote subsequent reward-seeking behavior. The role of BDNF, and its location of action given its antero- and retrograde transport, has not been assessed in this type of social decision making. Here we find that both neuronal and glial reductions in *Bdnf* within the MO impair the ability of mice to choose reinforcers based on prior social experience, even while they are dispensable for a non-social decision-making task requiring the MO. BDNF binding to its high-affinity receptor tropomyosin receptor kinase B (TrkB) within the MO is necessary for SIFC, given that overexpression of a dominant-negative TrkB in the MO and on neurons projecting from the MO to the basolateral amygdala (BLA) occlude SIFC performance. In addition, local *Bdnf* loss results in fewer mature dendritic spines on excitatory neurons within the MO but not on neurons within the BLA. Chemogenetic manipulations indicate that the MO contributes to SIFC by enabling mice to form associations between social experience and external reward. Ongoing investigations are evaluating whether BDNF signaling promotes SIFC through its regulation of excitatory neural activity in the MO.

Disclosures: T.T. Towner: None. **E. Seo:** None. **A. Leahy:** None. **E. McCall:** None. **K. Xing:** None. **T. Anand:** None. **S.L. Gourley:** None.

Poster

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Topic: I.03. Decision Making

Support: NSF NRT 2152260
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Title: A contributory role for the rat frontal orienting field (FOF) in withholding free response decisions

Authors: *K. STEWART[1], T. D. HANKS[1,2];
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Abstract: The ability to flexibly accumulate sensory evidence over time is essential for adaptive behavior. While the frontal orienting field (FOF) has been implicated as a brain region important for decision selection during perceptual decision making, few studies have investigated its role in free response decisions. Here, we probe FOF's role in decisions requiring dynamic evidence evaluation using a novel rodent change detection task. Adult rats were trained to detect increases in auditory click rates drawn from a stochastic Poisson process and to report those changes through an orienting movement. To test FOF's contribution to free response decision processes, we first used the GABA agonist muscimol to perform reversible pharmacological inactivation of FOF while rats performed the change detection task. Intriguingly, whole-session bilateral FOF inactivation led to increased decision responsiveness compared to control sessions. This suggests

an important role for FOF in withholding decisions during deliberation. To test the temporal specificity of these effects, we performed optogenetic inactivation of FOF during specific trial epochs while rats performed the same change detection task. We found that transient, bilateral inactivation of FOF could also increase decision responsiveness, specifically when the inactivation occurred later rather than earlier in the deliberation process. Psychophysical reverse correlation analyses of late trial inactivation revealed a significant change in the timing of stimulus features preceding detection reports, suggesting that FOF influences not just when animals commit to a choice but also how evidence influences that choice. Taken together, our results suggests that FOF contributes to shaping free response decisions with an active role in withholding decision commitment during deliberation.

Disclosures: K. Stewart: None. **T.D. Hanks:** None.

Poster

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Topic: I.03. Decision Making

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R01 Grant MH124818
UC Davis Large Interdisciplinary Applications in Neuroscience (LIAN) Planning Grant: Neuromodulatory control of decision-making

Title: From impulsivity to engagement: latent behavioral states modulate neural dynamics in perceptual decision-making

Authors: *A. AKHMETZHANOVA[1], A. GOLDRING[2], R. CHAUDHURI[3], T. D. HANKS[2]; [1]Ctr. for Neurosci., [2]Ctr. for Neuroscience; Dept. of Neurol., [3]Ctr. for Neuroscience; Neurobiology, Physiol. and Behavior; Mathematics, Univ. of California Davis, Davis, CA

Abstract: Neural dynamics and behavioral outcomes are highly variable, adapting to different stimuli, contexts, and events. This variability in neural activity and behavior is thought to arise in part from latent states associated with varying levels of arousal, motor activity, or attention. These latent states may critically shape how the brain encodes sensory inputs, guides decision-making, and generates observable behaviors. However, the extent to which neural and behavioral states interact to drive differential neural activity and behavioral outcomes remains unclear. In this study, we investigated the relationship between hidden behavioral and neural states during perceptual decision-making. We employed an auditory change-detection task in which rats were trained to report increases in the click rate of a Poisson-generated stimulus. During performance of the task, we used Neuropixels probes to record neural activity simultaneously from hundreds of neurons across two key decision-related brain regions: the frontal orienting fields (FOF) and the anterior dorsal striatum (ADS). To bridge behavioral and neural datasets, we applied a

Generalized Linear Model-Hidden Markov Model (GLM-HMM) to infer underlying states across both domains and explore their relationship. Our behavioral modeling allowed us to identify distinct behavior across multiple individual sessions. This included states associated with more impulsive decision making where rats respond more rapidly during the trials with a heavy bias for false alarm responses and other states with fewer false alarms and higher levels of performance. Analyses of neural activity showed that mean firing rates remained largely stable across the behavioral states. However, we found intriguing alterations of correlational structure based on underlying behavioral latent state, suggesting a possible shift in interregional coordination aligned with behavioral state transitions. Overall, our results highlight that state-dependent changes in decision-making behavior may involve coordinated network-level changes that are most identifiable with large-scale recordings.

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Poster

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Topic: I.03. Decision Making

Support: 2R01 DA047870

Title: Frontocortical circuit mechanisms for learning under stochastic and volatile conditions

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Abstract: In making decisions, the brain often has to arbitrate between multiple sources of uncertainty. For example, if the environment changes in its dynamics due to environmental volatility, a change in decision strategy may be necessary, whereas moment to moment stochastic variability should generally not be met with a change in strategy. Because both of these sources of uncertainty can underlie unexpected feedback for decisions, the brain must estimate the volatility of the environment separately from its stochasticity. We tested the contribution of two frontocortical regions, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC), to learning and decision strategy in an environment that is both stochastic and volatile. We trained rats to perform a “restless bandit” task in which subjects select between two choices, each associated with a different reward probability (0.80 vs. 0.20), with the reward probability of the choices alternating after a relatively low or high number of trials (μlow = 25, SD = 4; μhigh = 60, SD = 4). We first infused a virus containing inhibitory hM4Di DREADDs on a CaMK promoter targeted at rat ventrolateral OFC (n = 8) or ACC (n = 8). Then, we chemogenetically perturbed either ACC or OFC (via administration of 0.1 mg/kg

deschloroclozapine, DCZ) as rats transitioned to the higher volatility setting. We found that rats with ACC inactivation reduced adaptation to increased volatility, while OFC inactivation facilitated that adaptation according to block-averaged learning curves compared with vehicle control performance. In addition, we fit a novel Hidden Markov Model (HMM) with parameters for volatility and stochasticity to behavioral data with volatility settings depending on inactivation condition, and found that, while vehicle controls exhibited an increase in volatility but not stochasticity parameter upon transition to high volatility, ACC-inactivated rats did not exhibit this adaptation in volatility estimate. OFC inactivation, on the other hand, elicited a decrease in the stochasticity estimate, consistent with an amplification in adaptation. Our results suggest that ACC controls estimates of environmental volatility and thus adjustments in learning rate when that volatility changes, while OFC controls responses to recent environmental feedback irrespective of volatility.

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Poster

PSTR088: Prefrontal Cortex

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Topic: I.03. Decision Making

Support: NIH/NINDS R01NS135048

Title: Synaptic facilitation shapes flexible rule switching

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Abstract: Short-term plasticity (STP) dynamically regulates synaptic strength on the timescale of milliseconds to seconds, playing a critical role in moment-to-moment information processing within neural circuits. Deficits in STP have been implicated in neurological and psychiatric disorders, but its precise contribution to higher cognitive functions and behavioral flexibility remains poorly understood. Here, we address this gap by focusing on synaptotagmin-7 (SYT7), a calcium sensor essential for facilitation—a form of STP in which high-frequency stimulation briefly enhances neurotransmitter release. Using Syt7 knockout (KO) mice, we demonstrate that the loss of facilitation impairs flexible behavior while leaving baseline synaptic properties intact. Specifically, KO mice perform similarly to wild-type (WT) littermates on a short-term memory task, but are impaired when the reward contingency reverses from a “match” rule to a “non-match” rule, suggesting a selective deficit in cognitive flexibility. Neural recordings in frontal cortical regions reveal that KO mice exhibit subregion-specific disruption to low-frequency

oscillations evoked by reward outcomes, with a marked reduction in outcome-specific population coding in medial prefrontal cortex. While WT mice show distinct activity patterns for reward and punishment, these differences are absent in KO mice, indicating a disruption in encoding task-relevant outcomes. Finally, we use artificial neural networks to model performance in networks with and without synaptic facilitation. These models recapitulate both the behavioral inflexibility and neural coding impairments observed in KO mice, reinforcing the idea that synaptic facilitation plays a fundamental role in behavioral adaptation and flexible information processing. Our results establish a direct link between STP, prefrontal cortical function, and flexible behavior, while highlighting the computational power of synaptic dynamics in flexibly solving complex cognitive tasks.

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Poster

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Title: Efficient coding of a complex goal-directed behaviour in mouse medial-frontal cortex

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Abstract: Neural representations of the external world are often understood through the principles of efficiency - reducing redundancy of sensory input, and utility - supporting useful computations. However, it remains unclear whether, or how, these principles apply to neural representations of our own behaviour. Here we present data which suggests that medial-frontal cortex (mFC) contains an efficient code for the sequential structure of goal directed behaviours, which may support hierarchically organised action selection. We trained mice (Male, n=6; C57B6J) to navigate to visually cued goal locations in complex elevated mazes, and recorded neurons in mFC (prelimbic) using silicon probes (6-shank, 64 channel Cambridge Neurotech). We observed two prominent patterns in mFC activity: First, a population code for shortest path-distance to goal in which different neurons responded at different distances, such that the population tiled the navigation trajectory. Second, place-direction tuning in which neurons responded when subjects traversed particular sections of a maze in a given direction. This often

took the form of tuning to extended route segments, or motion towards bottle-neck locations, reminiscent of 'options' in hierarchical reinforcement learning (RL). The low-dimensional structure of this place-direction tuning across neurons spanned the same low-dimensional space as that of subject's behavioural trajectories across trials - suggesting they may comprise an efficient code for behavioural sequences. To quantify the encoding of place-direction, distance to goal, and their interaction, we developed a novel regression via neural latent embedding framework. This confirmed that each feature explained unique variance in the neural population, but in largely separate neural subspaces. Together, these results highlight the presence of distinct codes for goal-distance and commonly executed extended action sequences in mouse mFC during flexible navigation. These representations may be complementary, as work in machine learning suggests that sequence compression can discover useful options for hierarchical RL.

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Poster

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Topic: I.03. Decision Making

Support: 1U19NS123714-01

Title: Molecular, morphological, and projection-based organization of non-sensory thalamus

Authors: *M. T. SUMMERS[1], H. MYERS[1], B. OUELLETTE[1], N. LUSK[2], M. RUE[2], P. GROTZ[1], N. OUELLETTE[1], R. JAVERI[1], C. ARSHADI[1], S. TUGGLE[1], X. CHEN[2], A. G. CARTER[3], K. SVOBODA[1], J. V. CHANDRASHEKAR[1];
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Abstract: The thalamus is the primary long-range input to cortex, initiating and maintaining cortical dynamics underlying flexible behavioral switching. Thalamic connectivity to cortex has been studied at the level of cytoarchitectonic nuclei, but thalamocortical neurons within these nuclei are molecularly and structurally diverse. Here we take a multi-pronged approach to investigate the anatomical pathways by which signals are routed between thalamus and cortex with a focus on non-sensory thalamus. First, we use retrograde labeling and an automated whole-brain anatomy pipeline to comprehensively map projections to frontal cortex. We find subdivisions of thalamic nuclei organized by cortical output connectivity. Next, we use morphological reconstructions to study the axonal arborizations and collateralization of individual thalamocortical neurons, characterizing the diversity and topography of these projections. Finally, we use barcoded connectomics and *in situ* sequencing across an entire thalamic hemisphere to map the coarse organization of projections combined with gene expression of thousands of individual thalamic neurons. These Common Coordinate Framework

(CCF) aligned datasets provide an anatomical touchstone by which to understand the precise organization of thalamic drive to the cortex and enable distinguishing between discrete versus topographic organization of these outputs.

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Poster

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Biotechnology and Biological Sciences Research Council (UKRI-BBSRC) BB/T008784/1

Title: Transforming world to goal coordinates in the medial frontal cortex

Authors: *M. ZHU[1], Y. YANG[1], A. HARRIS[1], P. DOOHAN[1], M. E. WALTON[2], T. AKAM[1], T. E. BEHRENS[3], M. EL-GABY[1];
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Abstract: For decades, both the hippocampus and the prefrontal cortex have been recognized as key contributors to the construction of cognitive maps in the brain—one mapping the physical world (the world model), and the other mapping value and goals (the goal model). Previous findings from our lab and others have identified representations in the medial frontal cortex (mFC) that support goal model formation. This work has demonstrated that goal progress neurons in the mFC are basic building blocks for representations of complex tasks. How do brain circuits generate neuronal dynamics that evolve in relation to the animal's progress towards its goals, rather than time or distance? To answer this question, we trained animals on a multi-goal task, the "ABCD task", in which the animals navigate in a 3*3 maze and would need to visit four locations sequentially to obtain rewards. We find that, prior to a single run to a goal, mFC neurons represent the distance to the goal. Crucially, the error in decoding this prospective distance-to-goal signal is negatively correlated with that in decoding subsequent goal progress. This is consistent with the use of a goal-distance signal, derived from a world model, to normalise the velocity of an activity bump along mFC attractors in order to compute progress-to-goal in a manner invariant to the distance and sensory specifics of the route to goal. To test this model more directly, a causal manipulation is necessary. We hypothesised that the distance to

goal signal putatively used to normalise mFC dynamics may arise from the hippocampus, which is known to support world models. To investigate this possibility, we optogentically inhibited ventral hippocampus (vHPC) and examined how the manipulation affected the animals' behaviour and mFC representations in the ABCD task. Our results showed that vHPC inhibition impaired performance during the early phase of ABCD task learning but had little effect at later stages. Moreover, simultaneous recordings from the mFC that vHPC Inhibition shifted mFC tuning such that they over-estimated goal progress. Our analysis of data from a separate study where vHPC afferents were silenced in the mFC while mice performed a spatial working memory task also show an over-estimation of goal-progress when silencing vHPC inputs, consistent with our findings in the ABCD task. These findings suggest a learning-stage-dependent role of vHPC inputs in calibrating goal model attractors in the mFC, potentially pointing to complementary computational interactions between these key circuits.

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Poster

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Topic: I.03. Decision Making

Support: STI2030-Major Projects (2021ZD0203800)
NSFC32171036

Title: Neural mechanisms of abstract concept representation and generalization across frontal and parietal cortices.

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Abstract: Abstract concepts are fundamental to high-level cognition, yet the neural mechanisms underlying concept representation and generalization remain poorly understood. To address this, we recorded neural activity simultaneously across frontoparietal cortices in monkeys performing a delayed-match-to-concept (DMC) task. Subjects classified 26 diverse images (including photographs, abstract doodles, and oracle bone inscriptions) into "animal" or "plant" categories, demonstrating high accuracy and rapid generalization to novel stimuli, suggesting abstract concept formation. We recorded neurons across six frontoparietal subregions: frontal eye field (FEF), dorsolateral (dlPFC) and ventrolateral prefrontal cortex (vlPFC), ventral premotor cortex (PMv), lateral intraparietal area (LIP), and medial superior temporal area (MST). Concept and picture selectivity emerged in the vlPFC, FEF, PMv, MST, and LIP. Specifically, FEF and PMv showed stronger concept encoding than other recorded brain regions, while LIP exhibited

stronger and more sustained picture selectivity than the frontal regions. Those results indicate that the frontal lobe plays a dominant role in abstract information processing, while the posterior parietal cortex is more visual-feature driven. Notably, during generalization to novel stimuli, FEF and PMv neurons dynamically adjusted their responses as learning progressed, implicating the frontal cortex in concept generalization. Together, our results revealed how the frontal-parietal network transforms visual features into abstract concepts, advancing our understanding of the neural substrates underlying conceptual reasoning.

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Poster

PSTR088: Prefrontal Cortex

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Topic: I.03. Decision Making

Support: Data for this study were made available through CRCNS by L. Hunt and S. Kennerley.

Title: Prefrontal LFPs in non-human primates encode belief updating and reward processing during value-based decisions

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Abstract: *Objective.* Understanding the temporal and spectral dynamics of prefrontal cortex (PFC) activity is essential for decoding cognitive processes during decision-making. While local field potential (LFP) high gamma (Hγ) activity has been linked to local neuronal firing, its role in encoding belief updating, reward expectation, and outcome evaluation remains underexplored in primates. We investigated Hγ modulation in PFC subregions during a multi-cue reward-guided decision task. *Approach.* We analyzed LFP signals from chronic depth electrodes implanted in the anterior cingulate cortex (ACC), dorsolateral PFC (DLPFC), and orbitofrontal cortex (OFC) of two macaques performing a task where up to four sequential cues indicated the expected reward associated with different target options. High gamma power was extracted using sliding 0.5-second windows aligned to key task events. Support vector machine classifiers were used to decode belief states, reward expectation, cue identity, and outcomes. Channel-level analyses included p-value histograms, anatomical mapping, and modulation direction assessments. *Results.* High gamma power robustly discriminated between trials requiring belief updating (i.e., when the current cue changed the expected best option) and trials confirming prior expectations.

However, the direction of modulation varied between animals, suggesting individualized encoding strategies. Cue identity and position were also decoded from Hγ, especially in OFC and DLPFC, respectively. Around movement onset, Hγ distinguished between high and low expected rewards with up to 88.9% decoding accuracy. Additionally, Hγ modulated in response to reward omission, which is consistent with prediction error. The number and location of significantly modulated channels increased across session days, indicating learning-related plasticity. *Significance.* These findings highlight Hγ activity as a rich neural feature encoding belief dynamics and reward processing in prefrontal circuits. The subject-specific patterns underscore the need for individualized decoding strategies in cognitive-state BCIs. High gamma offers a reliable, spatially localized signal for tracking latent cognitive variables during complex decisions.

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Poster

PSTR088: Prefrontal Cortex

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Topic: I.03. Decision Making

Support: NIH Grant R01MH045573

Title: Frontal pole connectivity : neuroanatomy-guided tractography informs neuromodulation targets for psychiatric disorders

Authors: *M. CELESTINE[1,2], C. MAFFEI[3], J. LEHMAN[2], A. YENDIKI[3], S. N. HABER[2]; [1]Harvard medical Sch. - McLean Hosp., Belmont, MA; [2]Pharmacol. and Physiol., Univ. of Rochester, Rochester, NY; [3]Massachusetts Gen. Hosp., Cambridge, MA

Abstract: The frontal pole (FP, Brodmann Area 10) is a uniquely elaborated cortical region in the human brain, notable for its broad connectivity and cellular complexity. Positioned at the apex of the prefrontal hierarchy, it integrates information across distributed neural systems. Disruption of FP circuitry is increasingly recognized as a transdiagnostic feature in psychiatric illness, highlighting its potential as a neuromodulatory target. Despite this, FP connectivity is less understood than in other prefrontal areas. Given that stimulation coils and electrodes are often placed in rostral prefrontal cortex (PFC), clarifying FP connections is essential for linking stimulation to clinical outcomes. This study aims to define the unique nonhuman primate (NHP) neuroanatomy and structural connectivity of FP subregions and their relevance for neuromodulation. We performed bidirectional tracer injections in dorsal, ventral, and medial FP subregions in NHPs, along with adjacent areas including BA 9, BA 14, and anterior cingulate cortex (ACC). We quantified PFC and temporal cortical inputs and mapped frontopolar axonal projections. These anatomical data were used to guide diffusion MRI tractography in the same animals, enabling validation of tractography-based connectivity. We then extended the analysis

to healthy humans to assess conservation across species. Results revealed a distinct dorsoventral organization in FP projections: dorsal FP targets lateral PFC and medial temporal areas, while ventral and medial FP project to medial PFC and lateral temporal regions. These projection patterns mirrored the distribution of cortical inputs, suggesting reciprocal connectivity across most areas —except for the ACC. The FP strongly projects to the ACC but receives minimal return input, indicating a predominantly unidirectional, top-down influence. One FP subregion did show reciprocal ACC connections, supporting functional subdivisions within the FP. These distinctions were consistently replicated in both NHP and human tractography data. The conserved dorsoventral gradient in FP connectivity supports a revised parcellation of the human FP based on structural input-output profiles, with implications for neuromodulation. These findings provide new insights into FP organization and connectivity. Identifying distinct subregions with specific projection and input profiles informs more precise neuromodulation strategies. Aligning anatomical specificity with clinical relevance advances efforts to enhance therapeutic targeting in psychiatric disorders while minimizing off-target effects.

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Poster

PSTR088: Prefrontal Cortex

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Topic: I.03. Decision Making

Support: ASAP-020519
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UG3MH120094
ASAP-025193

Title: Linking cell types with circuits in the nonhuman primate brain via spatial transcriptomics

Authors: *O. R. BRULL[1], A. ALIKAYA[1], R. BHIK-GHANIE[1], R. K. TITTLE[1], M. FREDERICKS[1], M. HANADA[1], M. LIN[1], A. R. PFENNING[2], A. C. BOSTAN[1], W. R. STAUFFER[1];
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Abstract: Spatial transcriptomics is a cutting edge technique to examine single cell transcriptomes in anatomically preserved contexts and an ideal method to link molecular phenotypes with anatomical layers and circuits. The 10X Genomics Xenium Analyzer is a commercial spatial transcriptomics platform, which offers standard gene panels for detection of mouse and human genes. Our goals were to apply Xenium-based spatial transcriptomics to the nonhuman primate (NHP) brain, and to combine it with protein immunohistochemistry and

circuit tracing. We focused on the prefrontal cortex (PFC) and applied a conventional tracer - cholera toxin subunit-b (CTB) - to label inputs. We previously classified all excitatory and inhibitory neuron types in the rhesus macaque dorsolateral PFC (DLFPC) (He et. al 2025). Here, we leverage this non-spatial single cell data and Xenium-based spatial transcriptomics to reveal the spatial organization of the macaque PFC. We designed a custom Xenium panel by adding 99 rhesus macaque genes that complement the 254 genes in the predesigned human brain panel. We tested several tissue preparation protocols to optimize Xenium analyzer performance on 20-µm coronal sections from the macaque cortex. These methods included: 1) the direct mount (DM) method prescribed by the standard 10x protocol, 2) free-floating (FF) of sectioned tissue onto Xenium slides, 3) tape transfer (TT) using UV curing of tissue onto Xenium slides, and 4) a modified tape transfer (DM-T) approach without UV curing. The resulting data was analyzed using a combination of the Xenium Explorer, Seurat, and R. Overall, our analysis revealed that the DM-T approach resulted in the highest data quality, with DM and FF producing slightly lower quality of data. We segmented the cortical gray matter from each of the Xenium datasets, performed cluster analysis using R and Seurat, and utilized the Xenium Explorer to visualize the spatial organization of the resulting clusters. Cosine similarity revealed a high degree of consistency between clustering the Xenium platform and single-nucleus sequencing approaches (He et. al 2025). This supports the validity of our DM-T and FF approaches for using the Xenium platform. Using these techniques, we were able to visualize the distribution of the neuron subtypes - previously identified from single cell analysis of DLPFC - throughout the frontal cortex. We then combined spatial transcriptomics with immunohistochemical staining of CTB to unambiguously identify IT-projecting subtypes. Overall, our results establish a powerful method to link molecular phenotypes with circuits and pave the way for precise spatial targeting of circuit-breaking technologies.

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Poster

PSTR088: Prefrontal Cortex

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Program #/Poster #: PSTR088.22/GG3

Topic: I.03. Decision Making

Title: Dissociating Visual Discrimination and Confidence in the Prefrontal Cortex of Macaques

Authors: *X. YU[1], R. YAMAGUCHI[2], M. MITSUHASHI[2], H. LAU[4], T. ISA[3];
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Abstract: Understanding whether perceptual discrimination and confidence rely on shared or distinct neural mechanisms is critical for elucidating the basis of metacognitive evaluation. In

this study, we trained macaque monkeys to perform a four-alternative forced choice (4AFC) visual discrimination task with a post-decision wagering component. On each trial, monkeys reported the perceived location of a brief visual target by making a saccade, and then indicated their confidence by choosing between two options: a larger reward that required maintaining fixation for 2 seconds and was contingent on a correct response, or a small but guaranteed reward if the monkeys break fixation (before 2 seconds). Behavioral results showed that the fixation time after choosing the target location reflects confidence of seeing target. At the same time, we saw the dissociation between discrimination accuracy and confidence. In the fixation break trials, monkeys' correctness is still much higher than chance level, that is, discrimination accuracy does not parallel the monkey's confidence.To identify the neural basis of this dissociation, we recorded electrocorticographic (ECoG) signals from 48 electrodes implanted over the prefrontal cortex, focusing on the dorsolateral (dlPFC) and ventrolateral (vlPFC) regions. In high-confidence trials, low-frequency band activity (theta, alpha, and beta bands) in the dlPFC was significantly higher than in low-confidence trials, beginning shortly before target onset and peaking at around 180ms after the target presentation, suggesting a preparatory state conducive to metacognitive access. In addition, we observed a prominent increase in high-gamma activity (~70-150 Hz) in the vlPFC after target onset, peaking around 200 ms, which was also significantly greater in high-confidence trials.Overall, our study showed the task we used can reflect dissociation between discrimination and confidence. Neural activity results demonstrate a possible mechanism underlying this dissociation, offering novel insights into the dynamic architecture of metacognition.

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Poster

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Program #/Poster #: PSTR088.23/GG4

Topic: I.03. Decision Making

Support: UF1-NS122123

Title: Disentangling contextual-processing from sensory salience using population-level analyses of neural activity

Authors: *J. STEFANOWICZ[1], A. DUBEY[2], S. OSTOJIC[4], B. PESARAN[3];
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[4]Ecole Normale Superieure, Paris, France

Abstract: Rarely are the "rules" of naturalistic choice-behavior explicit and all immediate information useful. Context governs the rules that integrate sensory and goal-oriented information in complex cognitive functions. However sensory salience often distracts us,

impeding our ability to follow the context. Here, we use an innovative behavioral paradigm to study how sensory salience interferes with context-dependent processing of relevant information. We investigate the prefrontal cortex (PFC) mechanisms that disentangle context-dependent modulation from variations in sensory salience. We trained two rhesus monkeys on a two-target, free-choice luminance-reward selection task in which the rewarding target - either a horizontal or vertical bar - was varied in a block-design to allow for contextual association over trials. Target luminance was varied randomly per-trial independent of the context, thereby, sometimes acting as a distractor when in conflict with the context and sometimes in congruence with the context in an unpredictable manner. We recorded spiking activity (~200 units per monkey) in the PFC and employed targeted dimensionality reduction methods to identify dimensions of the population dynamics that encode different types of information. Discriminant analysis revealed weak - albeit significant - contextual encoding prior to choice in both monkeys. Consistent with prior work we confirmed the presence of context-dependent computations in PFC during a choice task. We observed that the two monkeys used different behavioral strategies in the presence of sensory salience. For one "deliberate" monkey, sensory salience did not interfere with context while for the other "reactive" monkey, sensory salience acted as a distractor. Comparison of the low-dimensional organisation of neural activity accounted for observed inter-subject behavioral differences. For the deliberate monkey, sensory salience aligned with stimulus information immediately following target onset. In contrast, for the reactive monkey sensory salience aligned with the choice dimension of neural activity. Our work demonstrates significant utility of the population analysis for uncovering nuanced neural dynamics - especially for complex tasks for which multiple "valid" strategies may exist and guide inter-subject analyses.

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Poster

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Topic: I.03. Decision Making

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Title: Spatially aware clustering of nonhuman primate dopamine neuron subtypes

Authors: *A. ALIKAYA[1], G. ABDELHADY[4], O. R. BRULL[1], M. HANADA[1], M. LIN[2], A. R. PFENNING[5], A. C. BOSTAN[3], W. R. STAUFFER[3];
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Abstract: The dopamine neuron cell groups A8, A9, and A10 are located in the ventral midbrain. These neurons are widely implicated in reward processing - the majority of dopamine neurons code for reward prediction errors: the differences between received and predicted rewards - but degeneration of dopamine neurons is linked primarily to the motor signs of Parkinson's disease. Recent studies have revealed dopamine neuron subtype heterogeneity in human, nonhuman primate (NHP), and rodent midbrains, and it is possible that these dopamine subtypes have different functions that explain the dual roles of dopamine neurons in reward processing and movement generation. Here, we used the 10X Genomics Xenium Analyzer and traditional immunohistochemistry to define spatial distribution of dopamine neuron subtypes in the macaque ventral midbrain. We performed spatial transcriptomics using the 10X Genomics standard human brain panel of 256 human genes and added a custom panel with an additional 99 rhesus macaque genes. Following the Xenium workflow, we verified dopamine neuron identity by tyrosine hydroxylase (TH) immunohistochemistry and aligned the imaged immunohistochemistry with the spatial transcriptomics. We used the xenium analyzer to indicate a region of interest (ROI) around the dopamine midbrain and then exported the data from cells within this ROI to Seurat. We used the standard Seurat single cell pipeline to cluster the data and then used known dopamine neurons marker genes, TH, DAT, and DRD2, to identify dopamine neuron clusters. We isolated these clusters and re-processed them with the Seurat pipeline. As with previous studies of dopamine subtypes, we found two main classes of dopamine neurons, one defined by the expression of CALB1 and the other by the expression of SOX6 and each class containing multiple subtypes. We used the Seurat pipeline to assign cluster identities to these subtypes and then aligned the identities with the Xenium workflow output. Our results showed clear spatial separation of these main classes and their subtypes, with CALB1+ neurons primarily located medially in the VTA and SOX6+ neurons located laterally in the ventral tier of the SNc. These results will aid in guiding cell-type specific manipulations of dopamine neurons in NHP, allowing for enhanced and direct control of underlying circuits known to contribute to complex behaviors.

Disclosures: A. Alikaya: None. **G. Abdelhady:** None. **O.R. Brull:** None. **M. Hanada:** None. **M. Lin:** None. **A.R. Pfenning:** None. **A.C. Bostan:** None. **W.R. Stauffer:** None.

Poster

PSTR089: Neurocognitive Flexibility Across Development

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR089.01/GG6

Topic: I.03. Decision Making

Support: 5R01MH127214-03
1F31MH138135-01

Title: Hardwired by hardship: a stress-sensitive vmPFC-BLA circuit fuels adolescent avoidance following early adversity

Authors: *C. M. GOODPASTER[1], M. GONGWER[2], L. A. DENARDO[2];
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Abstract: Early life adversity (ELA), such as childhood neglect or maltreatment, increases the risk of psychiatric disorders by heightening threat responses and impairing goal-directed behaviors—hallmarks of conditions including anxiety and depression. These conditions often emerge during adolescence, yet most animal research has focused on adult outcomes. Thus, the mechanisms connecting ELA to the development of threat sensitization remain unknown. Neural circuits linking the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA), which integrate threat and reward processing, are disrupted by ELA and implicated in psychiatric disease. Corticotrophin releasing hormone (CRH), a key stress-related neuropeptide, is also altered by ELA, but its role in shaping circuits underlying threat-induced behaviors remains unclear.To address this gap, I employ limited bedding and nesting (LBN), an ELA model, during the first week of life in mice. During adolescence, I combined a platform-mediated avoidance (PMA) assay with in vivo neural circuit investigations. LBN mice displayed increased avoidance behavior, which the BLA strongly encoded. Tissue clearing and lightsheet microscopy revealed a reduction in CRH+ cells in the ventromedial PFC (vmPFC) of LBN animals. Viral tracing and immediate early gene labeling uncovered that CRH+ vmPFC neurons project to the BLA and are hyperactive during PMA in LBN mice. Further, optogenetic inhibition of this pathway during PMA training significantly reduced avoidance behavior in male LBN mice, with no effect in females or controls. These findings reveal a novel, stress-sensitive vmPFC-BLA circuit that drives maladaptive avoidance following ELA, offering insight into how early stress alters adolescent brain function.

Disclosures: C.M. Goodpaster: None. **M. Gongwer:** None. **L.A. DeNardo:** None.

Poster

PSTR089: Neurocognitive Flexibility Across Development

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR089.02/GG7

Topic: I.03. Decision Making

Support: NIH Grant MH126178

Title: Examining the acute and persisting effects of adolescent SSRI administration on motivated behaviors

Authors: *C. FENGLER[1], K. M. NAUTIYAL[2];
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Abstract: Serotonin is a trophic factor, in addition to a neuromodulator, that influences neural development. Selective serotonin reuptake inhibitors (SSRIs) are a first line pharmacological treatment for major depressive disorder. They are also effective for treating adolescents, with two SSRIs (including fluoxetine (flx)) approved for age 12+. While adolescence is an inflection

point for the development of mood and anxiety disorders, it is also a period of maturation for the underlying neural circuits that are dysregulated in psychiatric disorders. The role of serotonin is not well understood during this developmental time period. Our study measures the acute and persistent effects of adolescent chronic SSRI treatment on motivation in mice.We administered flx (18 mg/kg/day) to mice via drinking water beginning on post-natal day (PND) 23 or PND 101, and trained them using a homecage closed-economy operant system to receive liquid rewards. After three weeks of flx administration, they were tested on random ratio (RR) and progressive ratio (PR) paradigms to measure motivation. After testing, animals were returned to their homecages for a 6 week washout period in which they consumed food and water ad lib. Following the washout, they were restested on RR and PR paradigms to measure the long lasting effects of adolescent or adult flx treatment on motivated behavior.During drug administration, flx-treated adolescent mice were more motivated than age matched controls, earning 24% more rewards across RR paradigms. Interestingly, this effect on increased motivation persisted after the washout period when the animals were retested as adults. To control for age, a second group of mice was treated with flx as adults; they too showed higher motivation, earning more liquid rewards during treatment compared to controls ($p < 0.001$). These effects also persisted after the washout period into later adulthood. Within-subject analysis revealed that motivation decreases from adolescence to adulthood, however flx-treated adolescents show more variable changes over time.Overall our findings suggest that flx increases motivation during treatment, but unexpectedly, these effects persist long beyond the termination of administration. This was the case in both adults and adolescents, suggesting that SSRI treatment during initial operant training/testing may influence reward value or action association learning, thus increasing responding upon retesting. Ongoing experiments will determine if initial testing during flx treatment influences later motivated behavior, and also evaluate the effects of flx on impulsivity and other reward-related behaviors.

Disclosures: C. Fengler: None. **K.M. Nautiyal:** None.

Poster

PSTR089: Neurocognitive Flexibility Across Development

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR089.03/GG8

Topic: I.03. Decision Making

Support: NIH Grant DA045913

Title: A distinct adolescent profile for dopamine-mediated neural signaling during Pavlovian conditioning

Authors: *E. HERRING[1], M. HAFENBREIDEL[2], E. PATEL[1], S. Y. ZENG[1], T. SYAMALA[1], M. M. TORREGROSSA[2], S. E. MORRISON[1];
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Abstract: Across species, adolescence is a time of heightened sensitivity to rewards, along with enhanced impulsivity, exploration, and risk-taking. In adults, some of these behavioral features are associated with a tendency towards "sign tracking": approach and interaction with a Pavlovian reward cue that is spatially separated from reward delivery. Other animals, in contrast, have a tendency towards "goal tracking," or approach to the site of reward. Sign tracking is thought to reflect the transfer of incentive salience from reward to cue. Perhaps surprisingly, we and others have shown that adolescents are less likely to perform sign-tracking behavior, and more likely to perform goal tracking, compared with adults. This led us to investigate the neural basis of this counterintuitive behavioral trend. Therefore, we recorded the activity of individual neurons in the nucleus accumbens (NAc) of adolescent rats (P35-42) during a Pavlovian conditioning task; in a subset of subjects, we recorded from the same individuals again after reaching adulthood. Then, in a separate experiment, we recorded dopamine release using a fluorescent indicator ($GRAB_{DA}$) in the NAc of adolescents and adults during Pavlovian conditioning.
As expected, we identified more adolescent goal trackers than sign trackers, and those who showed sign tracking as adolescents exhibited more vigorous sign-tracking behavior as adults. We noted that adolescents had lower NAc neural responses to both cue and reward at the start of training. While adolescent cue-evoked responses increased over the course of training and grew further in adulthood, reward-evoked responses peaked in adolescence and declined in adulthood, correlating with increased sign-tracking behavior. Meanwhile, when we examined NAc dopamine release, we found the expected robust differences between sign trackers and goal trackers, while adolescents mostly resembled their adult counterparts. However, notably, among adolescent sign trackers, there was relatively little cue-evoked dopamine release compared with adults. Overall, these results suggest that the behavioral evolution from adolescence to adulthood may emerge from a combination of changes in cue- and reward-evoked dopamine release and responsivity of NAc neural signaling to dopamine.

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Poster

PSTR089: Neurocognitive Flexibility Across Development

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR089.04/GG9

Topic: I.03. Decision Making

Support: K01-MH128800

Title: Early life adversity shapes sex-specific patterns of adaptive choice in adolescent rats

Authors: *M. RINCÓN-CORTÉS;
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Abstract: Early life adversity (ELA) profoundly influences brain and behavioral development (McEwen, 2003; Nelson and Gabard-Durnam, 2020). Yet, its effects on adaptive decision-making, including how these may differ by sex, remain poorly understood. To this end, we investigated how early life scarcity-adversity exposure shapes social behavior and cognition in adolescent rats, examining sex-specific patterns of adaptive choice across multiple behavioral domains. Male and female Sprague Dawley rats were subjected to a scarcity-adversity paradigm involving adverse maternal caregiving from postnatal days (PND) 2-9 (Lauraine et al. 2024, Porras et al. 2025). We then assessed social motivation using a social play test, problem-solving abilities using a progressive puzzle box task, and cognitive flexibility via odor-based rule shifting in adolescent rats. Our results show that ELA produced striking sex-specific alterations in adaptive choice strategies during adolescence. Specifically, ELA males exhibited reduced social play behavior as indexed by fewer dorsal contacts compared to control males (t-test: $p < 0.05$, n=9-10 per group), suggesting altered social reward processing during this typically social developmental period. Paradoxically, these same ELA males showed enhanced problem-solving in the puzzle box test, displaying shorter latencies to solve a subset of trials with increased physical challenges (2-Way RM ANOVA: $p < 0.05$, n=8-9 per group). In contrast, adolescent ELA females showed preserved social play but impaired cognition, as they required more trials to reach criterion and committed significantly more errors during simple odor-based rule shifting (t-test: $p < 0.05$, n=6-7 per group), suggesting reduced behavioral adaptability when environmental contingencies are changed. In ELA females, this cognitive impairment may suggest that ELA may differentially compromise prefrontal(PFC)-mediated executive function in females. Given the established role of dopamine (DA) D1 and D2 receptors in the PFC in regulating executive function (Floresco, 2013), ongoing studies are examining D1/D2 receptor levels within the medial PFC. In sum, our data suggest that ELA exerts sex-specific effects on social behavior and cognitive function during adolescence, which may be associated with alterations within the mesoprefrontal/mesolimbic DA system.

Disclosures: M. Rincón-Cortés: None.

Poster

PSTR089: Neurocognitive Flexibility Across Development

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR089.05/GG10

Topic: I.03. Decision Making

Title: Differential corticolimbic activation and developmental shifts in behavioral choice during spatial recognition memory tasks

Authors: *P. A. ROBINSON-DRUMMER;
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Abstract: The recognition memory tasks are well suited to examine the neurobiological ontogeny of spatial memory due to the protracted emergence of memory across task variations

during early life. Neural circuit recruitment changes as task complexity increases; those that require spatial memory integration with contextual or object-feature information recruit wider corticolimbic networks. Previous reports in adult animals have shown that object identity, object location, and integrated spatial-object memory depend on the perirhinal cortex, hippocampus, and medial prefrontal cortex (mPFC), respectively. However, little is known about whether these regions function similarly in recognition tasks during development. The standard Object recognition task (OR) requires object identity memory, while the object location (OL) task examines object spatial location. The object-in-place (OiP) task requires integrating both object identity and location into memory. Our lab used these tasks to examine short-term (5min) memory during development. In juvenile (PD28) rats, mPFC cFos activity increased after OiP learning, but not OR or OL, relative to controls. Also, a positive correlation between mPFC and hippocampal DG activity was only observed after OiP training. These patterns suggest that some elements of spatial coding during these tasks may be similar between juvenile and adult animals. We also manipulated the spatial features of the objects and measured short-term memory. Interestingly, during the OR and OL tasks, pre-weanling rats (PD21) choose between rearing in the open context and mounting on the objects, dependent on the spatial characteristics of the objects during training and the task itself. Although pre-weanling and adult animals showed similar patterns of learning (i.e. stronger spatial memory for objects with distinct spatial dimensions relative to radially symmetrical objects), this behavioral shift was not observed in adults. Our results demonstrate an ontogenetic change in behavior that is sensitive to elements of the spatial environment. While previous reports suggest that hippocampal spatial mapping may be strengthened during rearing bouts, the neural locus of this behavioral variation in juveniles is currently unknown. Additional tests will explore whether differences in afferent connections to the hippocampus underlie shifts in behavioral choice during spatial learning in young rats.

Disclosures: P.A. Robinson-Drummer: None.

Poster

PSTR089: Neurocognitive Flexibility Across Development

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Program #/Poster #: PSTR089.06/GG11

Topic: I.03. Decision Making

Support: NIH Grant 1F31DA063524-01A1
NIH Grant 1 R01 MH127850-01

Title: Revealing activity signatures of early life adversity in the basolateral amygdala driving risky decision making

Authors: *C. R. CODY[1], S. RAMIREZ[3], H. C. BRENHOUSE[2];
[1]Psychology Dept., [2]Psychology, Northeastern Univ., Boston, MA; [3]Boston Univ., Boston, MA

Abstract: Traumatic events experienced early in life can shift an individual's behavioral repertoire in adolescence, causing increases in risk seeking behaviors. Understanding the ways in which early life adversity (ELA) increases adolescent vulnerability for risk taking is essential for preventing maladaptive mental health outcomes. ELA can alter the developmental trajectory of neuronal activity in regions controlling these behaviors, including the basolateral amygdala (BLA), nucleus accumbens (NAc), and prefrontal cortex (PFC). For example, prior work from our lab has shown that ELA leads to hyper-innervation of glutamatergic BLA projections to the PFC in adolescence. The connections between these areas are responsible for assessing threat, responding to novel stimuli, assessing valence, and reward-mediated behaviors. The BLA is particularly poised to control risk-related behaviors and is also vulnerable to ELA related changes. Thus, examining the trajectory of ELA-induced activity changes in the BLA may uncover a targetable neural mechanism underpinning adolescent maladaptive behavior. Here we utilized a 20-day model of maternal separation (MS) ELA, in which pups are separated from their mothers for 4 hours a day from postnatal day (PND) 2-20. In Experiment 1, cFos levels were assessed in both control and MS-reared pups on the final day of MS exposure to assess MS-induced changes to neuronal activity in the BLA, NAc, and PFC. This study elucidates the time-course of both typical neuronal activity maturation, as well as activity changes induced by MS within these regions. In Experiment 2, BLA cells activated by MS rearing were tagged with an activity dependent viral vector on the final days of MS exposure. Animals then were tested in the risky decision-making test (RDT) during the adolescent period to assess changes to adolescent risk taking induced by MS rearing. On the final day of the RDT (PND 60), animals were euthanized and cFos expression was measured in the BLA, NAc, and PFC. The number of cFos+ cells was assessed to see how MS experience affected activity in these regions during times of risk. In the BLA, the percent overlap of risk-induced cFos+ cells and tagged MS-induced cFos+ cells was quantified to assess if the same cell populations overactivated by MS are overactivated during the RDT. We hypothesized that overactivation of the BLA during ELA exposure drives increased risk-taking behaviors in adolescence via long-lasting facilitation of activity in risk-related neuronal ensembles. These findings will guide future circuit manipulations to further the understanding of how ELA alters risky behaviors in adolescence.

Disclosures: C.R. Cody: None. **S. Ramirez:** None. **H.C. Brenhouse:** None.

Poster

PSTR089: Neurocognitive Flexibility Across Development

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Topic: I.03. Decision Making

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NIH R00MH119320

Title: Behavioral flexibility in adolescent decision-making during approach-avoidance conflict

Authors: *G. E. MAGALHAES[1], M. SOARES[2], S. PRABHU[2], H. C. MEYER[2];
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Abstract: Natural environments contain both opportunities to encounter reward and threats. Deciding when to approach or avoid stimuli is essential to survival. Research has investigated how adult rodents use previously learned information about threat and reward to drive decision-making during situations of affective conflict, i.e., situations where both appetitive and aversive stimuli are present. However, many questions remain regarding how these decision-making processes refine throughout development. During adolescence, the ability to regulate affect changes dramatically, with adolescents being highly sensitive to both threat and reward. Yet, it remains unclear how adolescents would use learned information about threat and reward to navigate affective conflict. To investigate this, we developed a modified version of the platform mediated avoidance task in which animals learn to avoid tone-signaled foot shocks by stepping onto a non-electrified platform. In our task, food-restricted (80%) adult and adolescent mice undergo four days of appetitive conditioning (A.M.) where a 20s light cue signals the availability of a food reward (condensed milk), and avoidance conditioning (P.M.), consisting of tone-shock pairings, which can be avoided by stepping onto a platform. Next, mice undergo a conflict session (Day 5), consisting of 20 simultaneous presentations of the light and tone ("conflict cues"), signaling the chance to approach the reward port *and* avoid the imminent foot-shock using the platform. Our findings show that adolescents spend more time in the reward port during appetitive conditioning than adults and display greater odds of engaging in reward-seeking behavior when presented with a conflict cue. However, this reward sensitivity does not impair threat avoidance, as adolescents avoid shocks at roughly the same rate as adults during the conflict session. While both ages initially acquire avoidance at similar rates, adults are more likely to avoid threat when presented with conflict cues, which may be partially driven by high levels of threat avoidance in adult females. These findings inform age differences in decision-making in dynamic environments and illustrate that sensitivity to threat and reward during adolescence may lead to more efficient navigation of affective conflict.

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Poster

PSTR089: Neurocognitive Flexibility Across Development

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Topic: I.03. Decision Making

Support: NIDA K01DA053438
NIMH R01MH126183

Title: Neural representations of reward-related memories shift across development

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Abstract: Rewards signal valuable information that may be important to remember. Our recent work suggests that reward enhances memory through differential engagement of cortical and subcortical circuits across development. However, few studies have examined how reward memories are represented in the brain, or whether these representations exhibit systematic changes across development. To address these knowledge gaps, in 89 fMRI study participants ages 8 to 25 years-old, we used representational similarity analyses to quantify and compare neural pattern similarity between high- and low-reward encoding and retrieval trials in key memory-related brain areas. We found that reward value was reflected in pattern similarity during encoding in ventral temporal cortex and in change in pattern similarity from encoding to retrieval in anterior hippocampus. Reward-sensitive anterior hippocampus representations also varied with age such that reward-motivated memory benefits were supported by stability of hippocampal representations in older participants and drift in hippocampal representations in younger participants. Moreover, ventral tegmental area activation during encoding was associated the tendency toward representational drift in anterior hippocampus observed in younger participants. Our findings demonstrate that rewards influence neural representations and that the representational schemes supporting reward-motivated memory shift with age.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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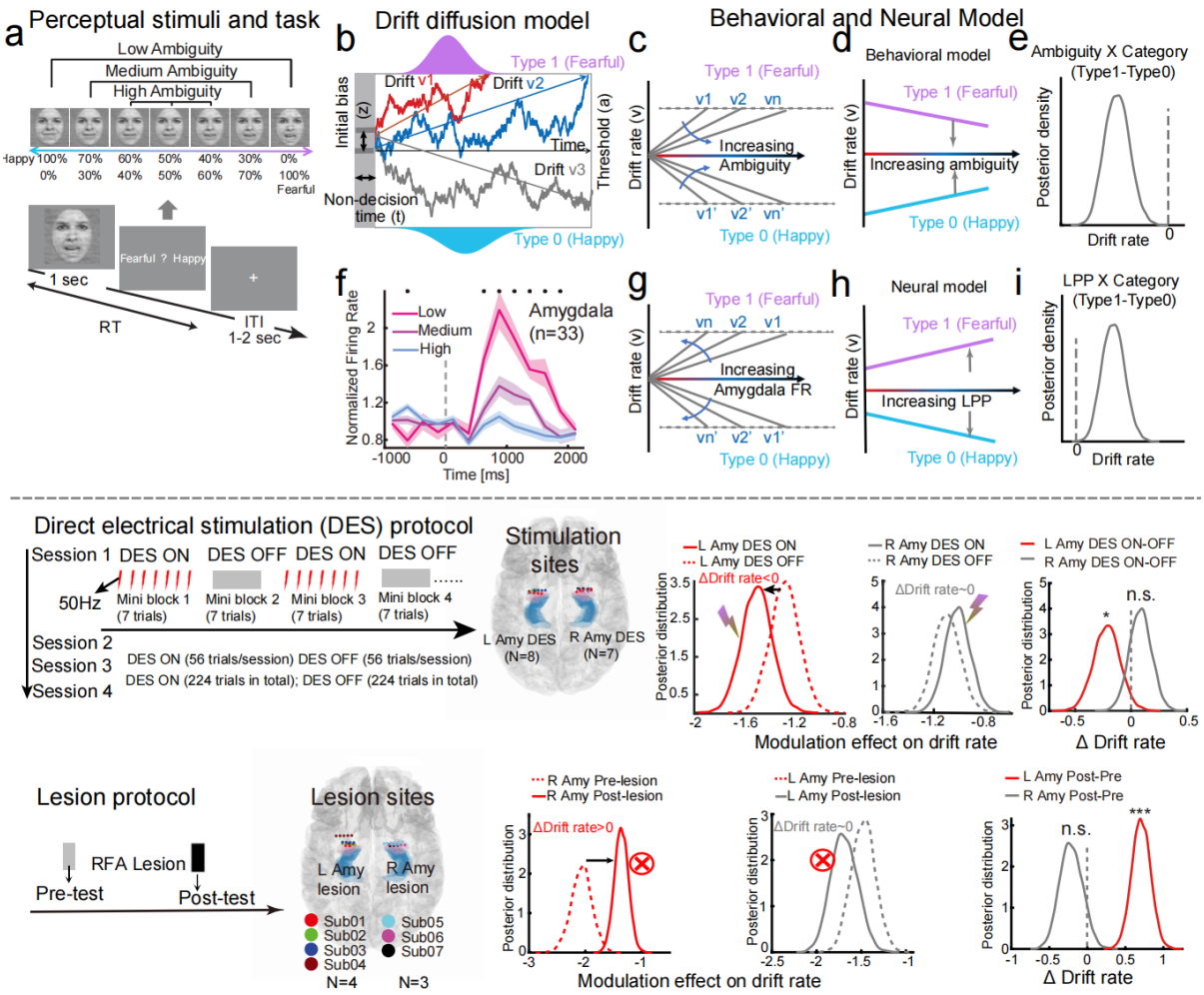
Topic: H.04. Emotion

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McDonnell Center for Systems Neuroscience
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Title: Neural and causal mechanisms of evidence accumulation in social-perceptual decision-making

Authors: *S. SUN[1,2], T. XIE[4], Y. CHEN[6], X. LI[7], R. YU[8], H. YU[9], P. BRUNNER[5], S. WANG[3], J. T. WILLIE[10];
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Abstract: Evidence accumulation has long been used to explain decision-making across various domains, including perceptual and higher-order social decisions. Yet, how evidence accumulation is gated in resolving perceptual ambiguity in emotional categories remains unclear. Previous theories have primarily focused on the roles of the prefrontal and parietal cortices in regulating this process across domains. Using a regression-based hierarchical drift diffusion model, we show that evidence accumulation efficiency, indexed by drift rate, declines as perceptual ambiguity in emotion increases across decision categories. Single-neuron recordings revealed that firing rates in the amygdala and dorsomedial prefrontal cortex (dmPFC) were modulated by drift rate. fMRI-based connectivity analyses further showed that dmPFC-amygdala coupling correlated with individual differences in evidence accumulation speed. To establish causality, we applied non-invasive electrical stimulation targeting the dmPFC, along with direct electrical stimulation (DES) and lesion methods targeting the amygdala. Anodal stimulation over the dmPFC and DES targeting the left amygdala increased evidence accumulation speed, whereas DES of the right amygdala had no significant effect. Ablation of the right amygdala, but not the left, attenuated the evidence accumulation rate. These findings support the view that evidence accumulation is dynamically gated by the dmPFC and amygdala, challenging the dominant notion that perceptual and cognitive speed is governed primarily by the frontoparietal cortex, and instead pointing to a broader neural circuit involved in social-emotional perceptual decisions, extending beyond previous theories.



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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR090.02/GG15

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NSF 2310626

Title: Understanding the role of the nucleus accumbens in distinguishing social preferences in the colonial spiny mouse (Acomys dimidiatus)

Authors: *A. I. BAUTISTA[1], H. BALASUBRAMANIAN[2], N. GOSE[1], M. MURUGAN[2], A. KELLY[1];
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Abstract: Spiny mice (Acomys dimidiatus) are gregarious rodents that form large mixed-sex groups in the wild. They readily affiliate with conspecifics, regardless of sex, novelty, or kinship status. However, we previously found that both males and females exhibit a robust preference for affiliating with male over female spiny mice. Whether this preference is rewarding or simply associated with salience is unknown. We therefore exposed spiny mice to distinct types of conspecifics while recording real-time calcium signaling in the nucleus accumbens (Nacc), an area of the brain important for processing reward, via wireless fiber photometry. We hypothesized that, if spiny mice find distinct types of social interactions differentially rewarding, thereby facilitating previously observed preferences, then males and females will exhibit greater neural activity in the Nacc when exposed to males compared to females. To test this, 6 adult male and 6 female spiny mice (PND 100-200) underwent stereotaxic surgery to receive a viral injection of GCaMP6F and a ferrule implant targeting the Nacc. Subjects were run in a time-blocked hour-long recording session, in which animals were exposed to novel opposite-sex and novel same-sex age-matched animals, or mealworms, interspersed with control (no interaction) blocks. Data was preprocessed using pMAT, and Bonsai was used to identify bouts of active investigation between the subject and stimulus animal. We then time-aligned investigative bouts to the average amplitude of GCaMP6f signal. Preliminary results demonstrate that calcium signaling in the Nacc is highest when males interact with a novel, same-sex individual, suggesting that nonreproductive interactions in males may be a more rewarding type of social interaction than interactions in a reproductive context. Additionally, in males, amplitude change occurred after physical contact with conspecifics, whereas amplitude change occurred prior to making contact with (i.e., on the approach toward) mealworms, indicating that spiny mice may differentially process reward in social and nonsocial contexts. These findings suggest that spiny mice may have evolved reward mechanisms to facilitate the drive to affiliate in nonreproductive contexts, enabling this species to thrive in large groups.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH Grant K99MH132772-01A1
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NARSAD Young Investigator Project # 00114844

Title: Sex differences in calcium signaling in the nucleus accumbens from dominant-subordinate social interactions in Syrian hamsters

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Abstract: The rewarding properties of social interactions are critical for the expression of adaptive social behaviors, and the development and maintenance of social relationships. There are also sex differences in social reward, which likely contribute to sex differences in psychiatric disorders. The nucleus accumbens (NAc) encodes reward for social behaviors and calcium is a biomarker of neuronal and synaptic activity. In the following study, I investigated changes in calcium signaling in the NAc in male and female Syrian hamsters with repeat dominant-subordinate social experience, i.e. the effects of social experience on calcium activity. I predict that with repeat social experience there is a potentiation in calcium signaling in the NAc, and this potentiation will be more pronounced in females. Adult male and female Syrian hamsters were single housed and injected with a virus containing a calcium indicator (AAV5-hSyn-Soma-jGCaMP8f) and a fiber optic cannula into the NAc. Four weeks later, hamsters underwent four days of testing in which they experienced a 5-min interaction with a stimulus hamster. Females were tested during diestrus. With repeat social experience, there is a decrease in the latency to initiate an aggressive interaction in females, a social behavior shown to be rewarding. There is also an increase in the calcium signal for the initiation of a social interaction, compared to just before the interaction, in males and females. As hypothesized, with repeat social experience there is a further potentiation in calcium signaling for the initiation of a social interaction compared to signaling during prior tests. Moreover, this potentiation is more pronounced in females compared to males. These studies support that social experience results in a potentiation in synaptic communication between neurons in the NAc in males and females. Further studies investigate the role of glutamate from the prefrontal cortex on this calcium signaling and social reward, and dissociate the role of the direct versus indirect circuit in the NAc in males and females. Advancing understanding of sex differences in social reward will advance understanding of sex differences in the susceptibility of psychiatric disorders.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Program #/Poster #: PSTR090.04/HH2

Topic: H.02. Reward and Appetitive Learning and Memory

Support: NIH R01-AG067011

Title: Characterizing influence of social closeness and subclinical depression on the link between striatal responses during reward sharing and processing of trust reciprocation

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Abstract: Social context shapes reward processing, with close others amplifying positive experiences. Yet, it is unclear whether the enhanced ventral striatal (VS) responses during reward sharing inform the processing of other social outcomes such as trust reciprocation. Further, depression, with its link to aberrant social valuation, could alter this association. In this ongoing preregistered study (https://osf.io/p654k), participants (n=91; ages 21-53, M = 32; 61 women) completed two fMRI tasks: the Card Guessing Game (Fareri et al., 2012), involving sharing monetary rewards/losses with a friend, stranger, or computer; and the Trust Game, involving investments in these partners followed by reciprocation or betrayal. We investigated whether VS activation during reward sharing with friends predicts the activation and connectivity of the neural substrates underlying social valuation during friend's reciprocation in the Trust Game. Preliminary results show that VS activation during shared reward predicted medial prefrontal cortex (mPFC) activation and default mode network (DMN) connectivity with mPFC, anterior insula, and superior parietal lobule during friend reciprocation. All these effects were only positive for lower friend closeness. Moreover, depression was associated with increased DMN connectivity with the mPFC, right frontal pole, and left cerebellum during friend reciprocation. Together, the result indicates that VS sensitivity to shared reward predicts enhanced engagement of social valuation circuits when interacting with less-close friends, but not with close ones, and suggests that VS activation reflects heightened neural responsiveness to uncertain, salient social interactions. Finally, depression appears to amplify DMN cross-network connectivity, potentially reflecting maladaptive attribution of social salience during reciprocation.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR090.05/HH3

Topic: H.04. Emotion

Support: Howard Hughes Medical Institute
Salk Institute for Biological Studies
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Dolby Family Fund
NIMH R01-MH115920
NIMH R37-MH102441
NCCIH Pioneer Award DP1-AT009925

Title: Sex-specific impacts of acute and chronic social isolation on social and valence processing in the mPFC

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Abstract: Reports of prosocial responses to acute social isolation and aggression following chronic social isolation led to a proposed conceptual framework for social homeostasis (Lee et al., 2021), but the behavioral and neural dynamics across social isolation remain unexplored. Previously, we found that dorsal raphe dopamine (DA) neurons mediate a loneliness-like state (Matthews et al., 2016) and innervate the medial prefrontal cortex (mPFC) (Lee et al., 2025). To explore how social isolation alters mPFC encoding of social and valence information, we used cellular resolution calcium imaging, genetically encoded DA sensors, ultrasonic vocalizations, and computer vision. Across durations (2 hours to 28 days) of isolation, adult male and female mice underwent a juvenile intruder task. Using DiscoPose, a custom SLEAP-based pipeline, we extracted pose features and applied unsupervised clustering to discover behavioral motifs. In males, isolation duration negatively correlated with interaction time (N=80 mice, r=-0.384, p=0.004), and 2 and 6 hours of isolation promoted social interaction with juvenile mice (N=107 mice, $F_{6,100}$=5.875, p<0.0001; GH vs 2hr: p<0.0001; GH vs 6hr: p=0.03). In contrast, isolation duration in female mice positively correlated with interaction time (N=54 mice, r=0.425, p=0.001), with increases after 14 and 28 days of isolation (N=63 mice, $F_{6,56}$=6.152, p<0.0001; GH vs 14d: p=0.022; GH vs 28d: p<0.0001). Unsupervised clustering of behavior features revealed changes in male social behavior, promoting face sniffing and reducing chasing in 7 and 28 day isolated mice (N=107 mice, $F_{18,400}$=3.705, p<0.0001), an effect not seen in females. Next, mPFC calcium imaging revealed that isolation increases the responsiveness of mPFC neurons to social contact (N=18 mice, n=3938 cells, GH vs 24hr: χ^2 =9.09, p=0.0106; GH vs 7d: χ^2=30.28, p<0.0001) by promoting excitatory responses (N=18 mice, $F_{2,15}$=5.189, p=0.019; GH vs 7d: p=0.015) in male mice, but not in female mice. Additionally, mPFC population trajectory length increased following isolation compared to prior group housing, an effect reversible followed by re-housing (N=6 mice, F2,6=16.60, p=0.004; GH_{D1} vs 24hr: p=0.011; 24hr vs GH_{D2}: p=0.006). To assess how social isolation alters valence processing, we trained mice on a Pavlovian discrimination task (N=8 isolated mice, N=4 grouped mice), enabling us to test the hypothesis that isolation-induced changes in mPFC social encoding co-occur with shifts in general valence processing. Together, these findings reveal sex-specific adaptations in mPFC processing underlying social and valence processing following social isolation.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR090.06/HH4

Topic: H.04. Emotion

Support: Salk Women & Science Career Development Award
Howard Hughes Medical Institute
Salk Institute for Biological Studies
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Dolby Family Fun
NIMH R01-MH115920
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Title: Investigating the Social Effects of Co-Stress: Co-Stress Increases Social Preference and mPFC Responsiveness

Authors: *J. CRESSY[1,2,5], F. ALOBOUDI[6], S. FLORES[2,5], S. MOHANTA[7,2], C. JIA[5], C. PARKER[2], A. SHAH[2], C. R. LEE[3,8], R. MIRANDA[2,5], L. KEYES[2,8], R. WICHMANN[2,8], K. M. TYE[4,5,9,8];
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Abstract: Social support profoundly shapes human health, reducing all-cause mortality by up to 45% (Wang et al., 2024). This effect is mediated by social buffering, a mechanism conserved across species where the presence of a social agent attenuates the stress response (Davitz & Mason 1955; Hennessy et al. 2000; Coe et al. 1978; Levine et al. 1978; Mendoza et al. 1978; Thorsteinsson et al. 1998). While stress in humans often occurs in social contexts, most preclinical models isolate animals during stress exposure (Godoy et al. 2018), limiting insight into how co-experienced stress influences future social behavior. To address this gap, we developed the Comparative Sociability assay to assess how shared stress influences social decision-making. In this paradigm, mice in the Stressed Together (ST) group co-experience inescapable footshocks. Changes in social preference are determined in a modified 3-chamber assay, where mice choose between interacting with their co-stressed partner or another cagemate. To examine how shared stress history shapes individual differences in neurophysiological responses during these social decisions, we simultaneously performed the behavioral assay,

episcope imaging of GCaMP8m-expressing neurons in the medial prefrontal cortex (mPFC), and peripheral hormone sampling within the same subjects. We compared the ST group to three control groups: Stressed Alone (SA), Unstressed Alone (UA), and Unstressed Together (UT), which controlled for individual stress exposure and social context. We hypothesized that only ST mice would develop a social preference for their co-stressed partner. Our results reveal a robust sex difference in post-stress social preference (N = 36 mice, p = 0.0005). A linear mixed-effects model confirmed that male, but not female, ST mice developed a preference for their co-stressed partner. Both sex (β = -77.58, SE = 19.40, t(68) = -4.00, p = 0.00016) and social stimulus (β = -83.99, SE = 18.29, t(68) = -4.59, p = 1.94e-05) significantly predicted changes in social preference. In vivo calcium imaging showed that co-experienced stress increased medial prefrontal cortex (mPFC) responsiveness to social interactions with the co-stress partner (N = 4 mice, n = 432 cells, p = 0.00013). Lastly, we use chronic venous access buttons to collect blood across the behavioral time course and quantify levels of corticosterone, testosterone, and progesterone via liquid chromatography-mass spectrometry (N = 4 mice). Together, this work introduces a novel method for studying co-experienced stress and uncovers sex-specific effects on social behavior, neural activity, and hormone regulation.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Program #/Poster #: PSTR090.07/HH5

Topic: H.04. Emotion

Support: National Science Foundation GRFP
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Salk Institute for Biological Studies
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Dolby Family Fund
NIMH R01-MH115920
NIMH R37-MH102441

Title: Changing social rank alters medial Prefrontal Cortex representations of positive and negative stimuli in social and non-social contexts

Authors: *R. MIRANDA[1,2], R. CASTRO[1], K. N. KIM[3], C. R. LEE[1], A. GARCIA[2], N. MOUSSA[2], C. JIA[2], K. BATRA[5], A. C. BASTUBA[1], R. SYED[2], L. R. KEYES[1,4], B. NIELSEN[1], R. WICHMANN[1,4], T. D. PEREIRA[1], K. M. TYE[1];
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Abstract: Many animal groups form social hierarchies, but the biological substrate underlying flexible representations of rank and how this influences valence processing is unknown. Previous work in our lab has shown that relative rank is represented in the medial Prefrontal Cortex (mPFC)(Padilla-Coreano 2022). The mPFC has also been shown to modulate social dominance, and forced loss to a subordinate has been shown to induce a depressive-like state (Fan 2023). To understand how social status affects valence representations, we designed a novel rank manipulation paradigm by rehousing mice of equal rank (e.g. dominants with dominants, subordinates with subordinates), forcing a rank change in a subset of mice. GCaMP7f was non-specifically expressed in the mPFC and imaged through a GRIN lens to track responses to positive and negative stimuli before and after rank change. During a Pavlovian Discrimination task, dominant mice exhibited more successful reward port entry. This behavior tracked changes in social rank, with new dominants seeing an increase in reward port entries. Change in social rank was negatively correlated with change in shock-associated mPFC inhibition, where drops in rank led to an increase in shock inhibition and increasing rank led to reduction in shock inhibition (N = 10 mice, n=2135 cells, p = 0.0008). Change in rank was also positively correlated with the number of mPFC neurons responsive to sucrose in the sucrose preference task (N=11 mice, n=2385 cells, p = 0.0013), suggesting a link between social status and valence representations.
To measure how rank influences social processing, we developed Peep Show, a novel assay for time-locked presentation of social agents optimized for neural recordings. Mice were presented with an aggressive male mouse, a female mouse in estrus, a male juvenile mouse, and an empty chamber in a trial-structured manner using photoswitchable glass. Change in social rank was negatively correlated with CD1-associated inhibition (N=9 mice, n=1382 cells, p=0.0112), and positively correlated Female-associated inhibition (N=9 mice, n=1382 cells, p=0.0108). Overall, our work provides a novel method for the manipulation of individual social rank, and reveals that changes in social status correlate with mPFC responsiveness to positive and negative stimuli in social and non-social contexts.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Program #/Poster #: PSTR090.08/HH6

Topic: H.04. Emotion

Support: Howard Hughes Medical Institute
Salk Institute for Biological Studies
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Kavli Foundation
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NIMH R01-MH115920

Title: Food Deprivation and exercise differently alter projection-specific valence-encoding populations in the basolateral amygdala

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Abstract: Studies have demonstrated that emotional state is warped by external stressors; however, not all stressors elicit the same effect. Exercise and food deprivation both increase caloric need, yet they induce positive and negative emotional states, respectively. How does our brain discern between internal states? Historical evidence suggests that the basolateral amygdala (BLA) is a nexus for emotional processing (LeDoux, 2000). BLA projections to either the nucleus accumbens (NAc) or the centromedial amygdala (CeM) preferentially encode positive and negative valence, respectively (Neih et al., 2015; Namburi et al., 2015). We endeavor to understand how projection specific populations in the BLA encode and process internal states, specifically exercise and food deprivation. We used a multi-photon imaging approach to track neural activity in mice trained on a Pavlovian discrimination task. We used SLEAP to track facial expression as an ethological measure of internal state change. In our discrimination task, mice were trained to respond to two audio cues (CS) which predicted either a positive (ensure) or negative (airpuff) outcome (US). We determined that food deprivation increases motivation through anticipatory licking ($P=0.0142$; $Df=32$) and that animals readily use exercise wheels. Food deprivation decreases spontaneous activity in BLA-CeM neurons ($P=0.0004$; $F=14.6$), whereas exercise increases spontaneous activity in BLA-NAc neurons ($P=0.03$; $F=11.62$). BLA-NAc neurons were also affected by internal state during both CS ($P<0.0001$; $F=31.35$) and US ($P<0.0001$ $F=65.68$) presentation; whereas. BLA-CeM neurons were affected by internal state during only CS presentation ($P<0.0001$; $F=39.15$), not US presentation ($P=0.42$; $F=69.42$). Neural trajectories reveal that food deprivation and exercise inhabit different dimensions in PC space, suggesting that one can decode homeostatic state from BLA population dynamics. These results suggest that projection-defined valence-encoding BLA populations are differently affected by internal state. Facial expression analysis demonstrate that mice anticipate positive and negative outcomes differently ($P=0.031$; $W=803$), and that internal state alters facial reactions to trial outcomes ($P=0.0471$; $dF=6$). Together, these findings suggest that the BLA modulates emotional processing with input from internal state by altering the tonic and phasic dynamics of distinct downstream circuits.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Title: Amygdalostriatal transition zone neurons encode sustained valence of stimuli to direct conditioned behaviors

Authors: *F. MILLS[1], C. R. LEE[2], J. R. HOWE[3], H. LI[4], N. KEISLER[6], S. SHAO[7], F. TASCHBACH[6], M. E. LEMIEUX[8], F. ALOBOUDI[9], J. WHITE[2], M. CHAN[10], M. BORIO[2], L. KEYES[2], H. CHEN[11], F. BUSHRA[12], G. SCHNEIDER[13], K. J. LEE[12], K. BATRA[9], R. PATEL[5], M. ASOKAN[14], J. DELAHANTY[15], C. CAZARES[6], C. R. HEYMAN[12], L. MAREE[2], R. WICHMANN[2], T. D. PEREIRA[2], M. K. BENNA[6], C. M. ROOT[6], K. M. TYE[2];
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Abstract: In order to respond appropriately to threats in the environment, the brain must rapidly determine whether a stimulus is important and whether it is positive or negative, and then use that information to direct behavioral responses. The amygdalostriatal transition zone (ASt) is anatomically poised to play a critical role in these processes by providing a shortcut between corticolimbic and basal ganglia circuitry to mediate behavioral responses to stimuli in parallel with the amygdala. Like the amygdala, the ASt receives converging sensory input from the thalamic and cortical pathways. However, the projections of the ASt are distinct from canonical outputs of the amygdala complex, and are integrated with striatal circuits involved in action selection. Despite this intriguing circuit connectivity, the function of the ASt is almost completely unknown.
From in vivo cellular resolution electrophysiology recordings, we find that ASt neurons show robust sustained responses to negative valence stimuli (Two-tailed t-test, $p = 0.0016$, n= 193 ASt neurons paired, 71 neurons unpaired), which are distinct from responses observed in adjacent amygdala and striatum (One-way ANOVA, $p=0.0008$, n= 193 neurons ASt, 212 neurons TS, 145 neurons CeA). We further show that photostimulation of the ASt is sufficient to drive freezing and avoidance behaviors (Two-way ANOVA, group x laser interactions, $p = 0.0023$, $p = 0.0186$,

n=8 mice ChR2, n=10 mice eYFP). Using single-nucleus RNA sequencing and in situ RNA labelling we generate a comprehensive profile of cell types and gene expression in the ASt, and find the ASt is genetically distinct from adjacent striatal and amygdalar structures (n=18-25 mice, >15000 cells per brain region, >50k unique molecular identifiers per cell). We also find that the ASt has a greater proportion of neurons expressing Drd2 than neurons expressing Drd1a (Chi-square test, p=0.0032, n=8 mice, >8 sections per group), a unique feature compared to other regions of the striatum. Using in vivo calcium imaging, we show that that this Drd2+ ASt neuron population robustly encodes stimuli of negative valence (Two-tailed t-test, $p < 0.0001$, n=9 mice 296 neurons paired, 6 mice 257 neurons unpaired), and in loss-of-function experiments find that optogenetic inhibition of Drd2+ ASt neurons causes a striking reduction in cue-conditioned fear responses (41% decreased freezing, Paired t-test, p=0.0377, n=8 mice NpHR, n=9 mice eYFP). Together, our findings identify the ASt as a previously-unappreciated critical missing link for encoding learned associations and directing behavior.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR090.10/HH8

Topic: H.04. Emotion

Support: Sanford Institute for Empathy and Compassion

Title: Rhythmic neural activity during perceived conspecific distress precedes prosocial behavior in the rat

Authors: *S. KOOIMAN[1], I. ZANE[2], S. NEALE[5], A. LEISCHING[3], C. R. HEYMAN[6], A. HUTTON[7], Y. ORTEGA[2], C. SHANG[2], L. M. RANGEL[4];
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Abstract: Throughout history, humans have pondered the origins of our prosocial nature. Although modern neuroscience offers tools that allow us to explore the biological mechanisms of prosocial action, systems-level electrophysiological investigations of helping behavior are relatively uncommon. To better understand the neural mechanisms involved in prosocial helping

behavior, we acquired behavioral (N=8; 6 Sprague-Dawley, 2 Long-Evans) and in vivo electrophysiological recording data (N=4) from female Sprague-Dawley rats engaged in a task where they had the opportunity to release a trapped conspecific (a cagemate, a stranger of the same strain, or a stranger from another strain) from an enclosed chamber. We found that subjects would alter their helping behavior based on the novelty of the social stimuli they encountered, with more frequent openings and reduced latency to open for more unfamiliar conspecifics. We then leveraged these differences in behavior to examine the temporal dynamics of neural activity within the anterior cingulate cortex and basolateral amygdala, brain regions associated with empathy and fear. We observed increases in the power of a 5-10 Hz rhythm and the entrainment of neural spiking activity to this rhythm as rats approached conspecifics in distress. Evaluating distributions of mean 5-10 Hz amplitude during all approach epochs revealed significantly higher power in both the ACC ($U = 1$, $p < .001$) and BLA ($U = 17$, $p < .001$) during approaches to a chamber containing a trapped conspecific compared to approaches to an empty chamber (two-tailed Mann-Whitney U test). 5-10 Hz power differences were also evident when comparing responses to different types of social stimuli. A trend in sustained 5-10 Hz mean amplitude activity was observed in sessions involving stimuli that were less likely to elicit helping behavior. In contrast, stimuli that induced helping behavior were associated with 5-10 Hz rhythm suppression in the opener rats (ACC: $U = 82$, $p = .17$; BLA: $U = 91$, $p = .047$). These findings collectively suggest that the observation of distress recruits 5-10 Hz rhythmic circuit activity in both the ACC and BLA, and the subsequent suppression of this activity coincides with increased helping behavior while its continuation inhibits prosocial action. The occurrence of 5-10 Hz rhythmic circuit activity thus serves as an important link between the perception of distress and the initiation of prosocial actions.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR090.11/HH9

Topic: J.07. Data Analysis and Statistics

Support: NIH NIDA-R34-DA061924

Title: Topological data analysis reveals stable relative postural states during social interaction between freely moving animals

Authors: *J. REILING[1], F. FROHLICH[3,4,5,6,7], M. ZHANG[1,2];
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Abstract: Capturing the rich, naturalistic dynamic behaviors is key to understanding social interaction in ecologically valid settings. However, common data analysis tools are tailored to task-centered paradigms. The growing field of topological data analysis (TDA) provides new tools to characterize the structure of complex data with greater intricacy, potentially better suited for analyzing naturalistic dynamics. Temporal Mapper is a TDA tool tailored for complex dynamical systems, which captures stable states (attractors) and transitions in complex dynamics in the form of a transition network. The nodes of the transition network represent stable behavioral states, and edges represent transitions between states. The nodes are weighted, where the weight reflects local stability of the behavioral state - how strongly it attracts nearby states. Recently, we developed a computational pipeline to construct transition networks from motion tracking data of naturalistic social interaction between two ferrets (*Mustela putorius furo*). First, we derived the relative posture between ferrets from the motion tracking data. The relative posture is depicted by the relative distance, angle, and velocity between body segments of the two ferrets. We then converted the relative posture time series into a transition network using Temporal Mapper. For comparison, we also constructed transition networks using single-ferret posture time series. To examine whether social and non-social behavior can be distinguished by posture dynamics, we compared the node weights during social interaction and non-social activity in both the relative postural transition network and the single-ferret transition network. In the relative postural transition network, we found significantly larger node weights during active social behavior compared to those of non-social behavior. That is, animals' relative postural states were more stable when they were socializing than when they engaged in non-social activities. In contrast, single-ferret posture transition networks exhibited no significant difference in node weights across social vs. non-social behavior. That is, a single ferret's posture states have the same stability across social vs. non-social behavior. Together, our findings suggest that social interaction is associated with stable relative postures between animals, which cannot be reduced to the postures of the individual animals. This project provides an initial step towards a unified computational tool for modeling naturalistic social interaction across the brain and behavioral scales.

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Poster

PSTR090: Neural Circuits Involved in Social Behaviors

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR090.12/HH10

Topic: H.04. Emotion

Support: Japan Science and Technology Agency (JST) - Moonshot Research and Development, Grant Number JPMJMS2295-03
Japan Society for Promotion of Science (JSPS), Grants-in-Aid for Scientific Research (Fostering Joint International Research(B), Grant Number 18KK0280

Title: A Predictive Model for Team Flow Based on Solo Flow Across Cognitive Abilities

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Abstract: Team Flow is a highly immersive, synchronized cognitive state linked to improved learning, engagement, and group performance. We previously identified its neural correlates using EEG hyperscanning during collaborative gameplay (Shehata et al., 2021). Building on this, we developed a hierarchical trait-state model to decode dyadic social interactions from EEG data (Wu et al., 2025), distinguishing stable neural traits from transient neural states. Together, these form individual "neural fingerprints," and individuals with more similar fingerprints are more likely to achieve synchronized Team Flow.To assess generalizability and enable predictive modeling, we turned to the Cattell-Horn-Carroll (CHC) theory of intelligence as a framework. We initially mapped 13 cognitive games across CHC domains and selected a simplified card-matching paradigm consisting of three variants: Object Match (visual processing), Word Match (crystallized linguistic knowledge), and Equation Result Match (quantitative reasoning). Each game was tested under "Go" (click leads to card flip) and "No-Go" (click does not flip cards) conditions, allowing modulation of task demands. By varying Go timing, we manipulated the task to induce adaptive flow, overload, or boredom conditions. Participants completed both solo and dyadic versions of each variant, with dyads alternating clicks in a turn-based sequence. Solo sessions occurred in separate rooms to prevent social priming.We recorded behavioral metrics (reaction time, error rates) and subjective flow experiences (Shehata et al., 2021). Preliminary results show that teams formed based on optimized solo flow and cognitive profiles exhibited superior performance and more aligned team flow subjective experiences than randomly assigned teams. These findings support the feasibility of building a predictive model of Team Flow, $p(TF) = f(SF, C, T, E)$, linking Team Flow (TF) to solo flow (SF), cognitive abilities (C), task features (T), and environmental context (E). Once these results are confirmed, we plan to collect EEG data to assess enhanced inter-brain synchrony. We hypothesize that this CHC-informed model enables scalable generalization to real-world collaborative tasks through building individual neural and cognitive fingerprint. Predicting and optimizing Team Flow based on individual cognitive abilities has significant implications for enhancing team performance in domains such as education, and workforce.

Disclosures: M.H. Shehata: None. **R. Shanavas:** None. **A. Yelland:** None. **S. Shimojo:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

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Program #/Poster #: PSTR091.01/HH11

Topic: I.08. Learning and Memory

Support: F31NS115572
R35GM124735
R01NS087544
P40 OD010440
R01DC005991

Title: Working memory in C. elegans emerges from internalized distributed motor command oscillators

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Abstract: Any animal navigating a complex environment stands to benefit from the ability to quickly form impressions of the world, retain them internally, and act on them later. But how did nervous systems acquire this foundational memory ability? Here, we report the existence of a working memory system in C. elegans that is employed for deferred action in a sensory-guided decision-making process.

During foraging worms make reversal-then-turn reorientation maneuvers, in either the dorsal or ventral direction, traditionally considered an unregulated random process (Pierce, 1999). However, fast olfactory neuron signaling is necessary for chemotaxis (Kato et al. 2014), suggesting worms may integrate sensory information with body posture to navigate effectively. We constructed a closed-loop virtual olfactory environment, combining a red-shifted depolarizing opsin, patterned illumination with a micro-mirror device, and real-time detection of head curvature (from crawling worms) or neural activity (during whole-brain imaging) to trigger optogenetic activation of the sensory neuron AWA during forward crawling, mimicking a temporally controlled asymmetric odor signal experience.

Animals showed a preference for resolving reversals with a turn in the favored direction, indicating (a) worms integrate olfactory sensation with proprioception or motor commands for headswings, and (b) to act appropriately after the reversal, the animal needs some form of working memory.

Previous studies showed that the worm brain cycles through broadly distributed, coordinated patterns of activity corresponding to major motor behavioral states; forward/reverse crawling and turning (Kato et al. 2015). We expand on this, finding that whole-brain dynamics decompose into two distributed oscillators, one for motor command state (i.e. forward/reverse) and another for head curvature (i.e. dorsal/ventral). We find that this working memory is implemented by the interaction of the command and headswing oscillators; AWA stimulation aligns their relative phase, which persists for 30-90s and through reversal, until producing a directed turn.

We propose that the original function of these oscillators was to produce physical movement, and they later evolved to support the function of working memory, endowing animals with the

ability to perform deferred sensory-guided action selection. This evolutionary step may represent the origin of a functional primitive of cognition.

Disclosures: R.L. Dunn: None. **C. Costello:** None. **N.D. L'Etoile:** None. **S. Kato:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Topic: I.08. Learning and Memory

Support: JSPS KAKENHI JP18H05213
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The Uehara Memorial Foundation

Title: Offline prefrontal cortex activity supports odor-based transitive inference emergence in mice

Authors: *W. H. MARCUS[1,2,3], M. NOMOTO[1,2,3,4], E. MURAYAMA[1,2,3], K. YAMADA-NOMOTO[1,2,3], K. INOKUCHI[1,2,3];
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Abstract: Transitive inference (TI) is a cognitive process of deductive reasoning that involves concluding relationships between items in the absence of direct evidence. Not only is TI reasoning capability exclusive to humans, but it has also been observed in several species, including primates, rodents, pigeons, and fish. In addition, previous studies suggested that brain activity during sleep, the idling brain, exhibited a crucial role in inference emergence. Despite the numerous reports of TI, the neural mechanisms underlying how the brain, especially during sleep, processes and organizes individual facts into structured knowledge remain quite elusive. To address this question, we have developed a novel behavioral paradigm for TI utilizing olfactory cues in head-fixed mice, under controlled sensory inputs. In this paradigm, mice initially underwent an olfactory operant Go-No-Go discrimination task. Afterwards, they were trained with four premise pairs: A>B, B>C, C>D, and D>E. Notably, they discriminated between the premises while adapting to the subsequent rule updating. Furthermore, mice displayed a preference for odor “B” over odor “D” when exposed to the “BD” transitive inference pair, akin to the “AE” non-transitive inference pair. Chemogenetic inhibition of idling activity after randomization in prefrontal cortex (PFC), but not hippocampus, impaired “BD” transitive

inference. Our results showed that head-fixed mice successfully constructed the entire hierarchy: A>B>C>D>E, demonstrating their ability to exhibit inferential behavior and that idling brain state, including sleep, may be important for such cognitive behavior. Currently, we are going to conduct a sleep-stage-specific optogenetic manipulation in the PFC to specify crucial sleep stages for our task. We are also going to perform Neuropixels 2.0 electrophysiological recording from the prefrontal cortex across different awake-sleep states to understand the inference-related neural representations. Taken together, our behavioral paradigm enables us to understand the neural basis of TI. Our goal is to elucidate the role of neuronal activity during sleep in intricate cognitive processes underlying biological intelligence, potentially advancing our understanding of analogous processes in diverse species, including humans, and it could help us to realize the general artificial intelligence.

Disclosures: W.H. Marcus: None. **M. Nomoto:** None. **E. Murayama:** None. **K. Yamada-Nomoto:** None. **K. Inokuchi:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Program #/Poster #: PSTR091.03/HH13

Topic: I.08. Learning and Memory

Support: NIH Grant R01NS116594

Title: Prefrontal gamma oscillations and task representations in a mouse rule shifting task

Authors: *C. COSTELLO[1], V. S. SOHAL[2];
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Abstract: In dynamic environments, animals must flexibly alter their behavioral strategies. The updating of behaviorally relevant associations supports rapid learning when environmental contingencies change. We study how the mouse brain implements this cognitive flexibility using a rule shifting task, in which mice sample combinations of odor and texture cues and learn to associate one cue with a reward. After mice learn this initial cue-reward association, the task rule changes such that a cue from the previously uninformative cue dimension becomes rewarded. Rule shift learning is known to depend on the synchronized gamma-frequency (~30-90 Hz) activity of parvalbumin-expressing inhibitory neurons (PVINs) in the medial prefrontal cortex (mPFC). Gamma-frequency stimulation of PVINs delivered out-of-phase between left and right mPFC hemispheres induces perseveration on the outdated rule. In contrast, the same stimulation delivered in-phase does not disrupt learning in otherwise normal mice, and it rescues learning in mutant mice that are perseverative. Although the mPFC is known to represent values, actions, and outcomes to facilitate flexible, goal-driven behavior, little is known about how the mPFC represents behaviorally-relevant information during this rule shifting task, or about how

interhemispheric gamma synchrony interacts with these representations to facilitate learning. Here, we use silicon probes to record from the mPFC during the rule shifting task. We find spiking that correlates with task-relevant actions, outcomes, and cues. We see an increase in gamma bursts (transient increases in gamma power) during unexpected negative outcomes after the rule changes. Units with significant spike-LFP phase locking to gamma bursts exhibit selectivity for a variety of task features. During bursts, the phase of firing for ensembles that encode the cue that was rewarded under the old rule predicts whether this encoding will persist when the new rule is learned. To characterize the effects of disrupting inter-hemispheric synchrony on neural dynamics and task encoding, we optogenetically drive gamma oscillations either in-phase or out-of-phase between mPFC hemispheres. When gamma synchrony between mPFC hemispheres is disrupted, mice spend more trials following the old rule, and the encoding of old-rule-relevant cues becomes more strongly maintained even after the rule changes.

Disclosures: C. Costello: None. **V.S. Sohal:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

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Program #/Poster #: PSTR091.04/HH14

Topic: I.08. Learning and Memory

Support: NIH / BRAIN Initiative Grant R01NS116594

Title: The insula and mPFC interact via gamma oscillations to promote cognitive flexibility

Authors: *L. L. HAGOPIAN[1], A. J. PHENSY DOS SANTOS[2], V. S. SOHAL[3];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Psychiatry, Univ. of California, San Francisco, Foster City, CA; [3]Dept. of Psychiatry, U. California, San Francisco, San Francisco, CA

Abstract: In many cases, the ability to shift behavior based on environmental cues has been shown to depend on the medial prefrontal cortex (mPFC). Our laboratory tests this ability in mice using a rule shifting task in which mice must learn to shift between texture and odor-based rules in order to find a hidden food reward (Cho et al., Neuron, 2015). Gamma-frequency activity in parvalbumin (PV) interneurons normally synchronizes between the left and right hemispheres when mice learn a rule shift, and disrupting this synchronization hinders the ability to learn a rule shift and causes perseveration (Cho et al 2020). However, the relationship of other brain regions to mPFC gamma synchrony is unclear. The insula, known for its potential involvement in cognitive and gustatory function, has bidirectional connectivity with the mPFC (Gogolla 2017). We found that bilateral optogenetic inhibition of mPFC-projecting insula neurons causes significant impairment when mice perform rule shifts specifically from texture-based rules to odor-based ones (number of trials needed to reach the learning criterion = 23.3 ± 6.9 in eNpHR-expressing mice vs. 16.5 ± 5.3 in controls, $p < 0.05$, $n = 18$). We have found no

significant impairment for odor to texture shifts or optogenetic inhibition during an odor initial association, concluding that the insula to mPFC projection is specific for odor rule shifts. This suggests that upstream regions may play sensory cue modality-specific roles in rule shifting. Furthermore, using Transmembrane Electrical Measurements Performed Optically (TEMPO), we have found gamma synchrony between PV interneurons in the insula and mPFC. These results show that insula forms a network with the mPFC that is defined by gamma synchronization and serves to facilitate changes in behavior that depend on odor cues.

Disclosures: L.L. Hagopian: None. **A.J. Phensy dos Santos:** None. **V.S. Sohal:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR091.05/HH15

Topic: I.08. Learning and Memory

Support: SFARI sex differences collaboration

Title: Sex Differences in the prefrontal encoding of social interactions in Shank3 knockout mice

Authors: T. BULLOCK[1], R. MALIK[3], *V. S. SOHAL[2];
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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;} Shank3 knockout mice have been used to study mechanisms underlying cognitive and behavioral deficits seen in Autism Spectrum Disorder (ASD). Previously, Shank3 knockout mice have been shown to have excessive neuronal recruitment, deficits in neuronal coactivity and correlations, and differences in neural ensemble recruitment and tuning during social behavior (Frost et al., PLoS Biology, 2021). Here, we explore sex differences in social encoding in the Shank3 knockout model with the goal of identifying potential neural mechanisms underlying the female protective effect, whereby females are less likely to manifest symptoms of and be diagnosed with ASD. To achieve this, we recorded medial prefrontal cortex (mPFC) activity in-vivo during various social assays using microendoscopic calcium imaging. We then trained neural network classifiers (NNC) to decode social behavior from calcium imaging data, and examined weights within the trained network to identify neuronal ensembles which encode specific social interactions. To examine the

generalizability of social encoding, we trained NNCs during interactions with one conspecific, then tested its ability to detect periods of social interaction during interactions with a different conspecific. Relative to wild-type males, Shank3 knockout males show a significant decrease in classifier generalization, but this effect was not seen in females. We are currently examining changes in neural activity levels, how these changes may be driven by differences ensemble of neurons that are positively and negatively modulated during social behaviors, and how these differences may contribute to the tuning of individual neurons within these ensembles.

Disclosures: T. Bullock: None. **R. Malik:** None. **V.S. Sohal:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Program #/Poster #: PSTR091.06/II1

Topic: I.08. Learning and Memory

Support: R01NS116594
NARSAD YI Grant #31117

Title: Circuit-specific synchronization of gamma oscillations between prefrontal PV interneurons and thalamic-projecting neurons supports flexible behavior

Authors: *A. J. PHENSY DOS SANTOS[1], L. HAGOPIAN[2], C. COSTELLO[4], V. S. SOHAL[3]; [1]Psychiatry, Univ. of California, San Francisco, Foster City, CA; [2]Psychiatry, [3]Dept. of Psychiatry, Univ. of California, San Francisco, San Francisco, CA; [4]Univ. of California San Francisco, San Francisco, CA

Abstract: Cognitive dysfunction in psychiatric disorders is often rooted in disrupted communication between the prefrontal cortex (PFC) and interconnected brain regions such as the mediodorsal thalamus (MD). Within the PFC, parvalbumin-positive interneurons (PVIs) play a pivotal role in regulating microcircuit dynamics and generating gamma-frequency oscillations, which can selectively entrain relevant neuronal ensembles while suppressing irrelevant activity. Our lab has previously shown that cross-hemispheric gamma synchrony among prefrontal PVIs is necessary for mice to shift behavioral strategies in a rule-shift task. However, how this synchrony influences downstream neuronal activity remains unclear. Here, we utilize single- and dual-color genetically encoded voltage indicators (GEVIs) with optogenetics to show that PVIs drive gamma synchrony specifically in a subpopulation of PFC projection neurons targeting the MD thalamus, supporting flexible decision-making. Notably, PVIs and MD-projecting neurons form dynamic synchrony states whose phase relationships shift across task epochs and reconfigure with learning. Although PVIs are necessary for the emergence of synchrony in these MD-projecting neurons, peak synchrony between the two populations occurs at distinct time points during the task, suggesting a temporally evolving coordination tuned to cognitive demands. In contrast, neighboring PFC neurons projecting to the dorsal striatum do not exhibit

these synchrony dynamics, underscoring the ability of PVIs to selectively entrain task-relevant subcircuits. Together, these findings demonstrate that the PFC engages gamma synchrony through flexible, cell-type- and projection-specific channels to support adaptive behavior. Our results highlight dynamic synchrony states as a candidate mechanism for coordinating information flow across PFC-thalamic networks during learning and behavioral flexibility.

Disclosures: A.J. Phensy dos Santos: None. **L. Hagopian:** None. **C. Costello:** None. **V.S. Sohal:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR091.07/II2

Topic: I.08. Learning and Memory

Support: NIH T32

Title: The role of prelimbic GABAergic interneurons in reward learning

Authors: *H. T. FRANKS[1], K. A. CUMMINGS[2];
[1]Univ. of Birmingham, Alabama, Birmingham, AL; [2]Neurobio., Univ. Alabama at Birmingham, Birmingham, AL

Abstract: Dysfunction in reward learning is a central feature of addiction. Most addiction studies focus on the role of dopamine and glutamate; however, dysfunction of cortical GABAergic signaling is also central to this pathology. In rodents the prelimbic cortex (PL) is a critical reward-encoding region. While the two most abundant PL interneuron subtypes, parvalbumin- (PV-INs) and somatostatin- (SST-INs) expressing interneurons, are central to other types of learning, the role of these interneurons in reward learning remains unstudied. I hypothesize that dynamic activity/plasticity of PL interneurons underlies reward encoding. To test this hypothesis, we subjected mice to fixed ratio-1 operant sucrose conditioning. 24 hours post-training, we performed *ex-vivo* slice electrophysiology and observed that SST-INs exhibit increased excitability following reward conditioning compared to naïve animals. In line with increased excitability, c-fos quantification after reward conditioning revealed a significantly higher proportion of c-fos+ SST-INs in animals learning the task compared to those that did not. Finally, *in-vivo* calcium imaging of PL SST-INs and PV-INs during reward conditioning revealed that both SST-INs and PV-INs exhibit slight increased activity during cue presentation. When pressing lever to receive reward, however, we observed that SST-INs and PV-INs exhibit increased and decreased activity, respectively. Intriguingly, both SST-INs and PV-INs exhibit learning-dependent activity alterations during cue presentation and lever press, indicating a novel role for PL interneurons in reward learning. Additionally, by utilizing cell-type-specific optogenetic manipulation, we discovered that inhibiting SST-INs, but not PV-INs, during lever extension led to a significant decrease in lever presses for reward, further exemplifying their role

in reward learning. Aside from underscoring the significant role of PL interneurons in reward learning, our results lay the groundwork for studying how PL interneuron dysfunction might contribute to maladaptive reward states, such as addiction.

Disclosures: H.T. Franks: None. **K.A. Cummings:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR091.08/II3

Topic: I.08. Learning and Memory

Support: 1ZIADA000587

Title: Prior cocaine use disrupts identification of hidden states by single units and neural ensembles in orbitofrontal cortex

Authors: *W. ZONG[1], L. MUELLER[2], Z. ZHANG[3], J. ZHOU[5], G. SCHOENBAUM[4]; [1]NIH, NIDA IRP, Baltimore, MD; [2]Psychology, UCLA, Los Angeles, CA; [3]Nation Inst. on Drug Abuse, NIDA IRP, Baltimore, MD; [4]NIDA IRP, BALTIMORE, MD; [5]Chinese Inst. for Brain Res., Beijing, China

Abstract: The orbitofrontal cortex (OFC) is critical to identifying task structure and to generalizing appropriately across task states with similar underlying or hidden causes. This capability is at the heart of OFC's proposed role in a network responsible for cognitive mapping, and its loss can explain many deficits associated with OFC damage or inactivation. Substance use disorder is defined by behaviors that share much in common with these deficits, such as an inability to modify learned behaviors in the face of new or even anecdotal information about undesired consequences. One explanation for this similarity would be if addictive drugs impacted the ability of OFC to recognize underlying similarities - hidden states - that allow information learned in one setting to be used in another. To explore this possibility, we trained rats to self-administer cocaine and then recorded single unit activity in lateral OFC as these rats performed in an odor sequence task consisting of unique and shared positions. In well-trained controls, we observed chance decoding of sequence at shared positions and near chance decoding even at unique positions, reflecting the irrelevance of distinguishing these positions in the task. By contrast, in cocaine-experienced rats, decoding remained significantly elevated, particularly at the positions that had superficial sensory differences that were collapsed in controls across learning. A tensor component analysis showed that this effect of reduced generalization after cocaine use also extended across positions in the sequences. These results show that prior cocaine use disrupts the normal identification of hidden states by OFC.

Disclosures: W. Zong: None. **L. Mueller:** None. **Z. Zhang:** None. **J. Zhou:** None. **G. Schoenbaum:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Program #/Poster #: PSTR091.09/II4

Topic: I.08. Learning and Memory

Support: Fi2 GM133534
K99 DA053633
ZIA DA000587

Title: Outcome-specific devaluation spontaneously recovers latent taste-predictive information in orbitofrontal cortex ensemble activity

Authors: *E. E. HART[1], L. BAHENA[2], G. SCHOENBAUM[3];
[1]Univ. of Maryland, Col. Park, College Park, MD; [2]Emory Univ., Atlanta, GA; [3]NIDA IRP, BALTIMORE, MD

Abstract: The orbitofrontal cortex (OFC) is critical when the relevance of previously acquired information changes, yet most single-unit recording studies focus on representation of information during stable task performance after extensive training. Under these conditions, the OFC encodes task-relevant information, generalizing across irrelevant sensory or other features. How does this transformation happen and what happens to irrelevant information that might be necessary later? Here we explored these questions by recording single-unit activity in rats learning an odor discrimination task in which odors were associated with different responses and flavored rewards. Activity evolved to represent task-relevant information in a format that generalized to new problems. However, irrelevant taste information remained latent in the activity and was recovered spontaneously upon selective satiation. The results provide insight into how the OFC organizes information and suggests this area is often necessary for adaptive behavior because of an ability to maintain irrelevant information for later use.

Disclosures: E.E. Hart: None. **L. Bahena:** None. **G. Schoenbaum:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR091.10/II5

Topic: I.08. Learning and Memory

Title: The anterior cingulate cortex dynamically encodes the valence of a conditioned stimulus in mice.

Authors: *P. GOSWAMEE[1], J. P. BURKETT[2];
[1]Anat. and Neurobio., Virginia Commonwealth Univ., Richmond, VA; [2]Neurosciences, Univ. of Toledo, Toledo, OH

Abstract: Fear is crucial for survival, enabling organisms to form learned associations between environmental cues and potential threats, but dysregulation of fear processing can lead to anxiety disorders and PTSD. Substantial evidence supports the involvement of the hippocampus and basolateral amygdala (BLA) in the storage of the fear engram, but the role of the anterior cingulate cortex (ACC), especially during and immediately after fear acquisition, remains poorly defined. To address this gap, we longitudinally recorded ACC neuronal activity using single-photon calcium imaging in freely behaving adult male mice subjected to a fear conditioning paradigm. Subjects acquired a conditioned freezing response to a tone cue (conditioned stimulus, CS) paired with light foot shocks (unconditioned stimulus, US), and ACC neuron activity was monitored during context pre-exposure, cue pre-exposure, fear acquisition, fear recall, and fear extinction. Consistent with known functions of the ACC, neuronal responses were modulated by the US and the novelty of the CS. Critically, both the number of CS-responsive neurons and the CS-associated population activity rose during fear acquisition, peaked following offline consolidation, and decreased over the course of extinction. Neuronal populations responsive to the CS throughout acquisition and recall overlapped at a rate consistent with chance. Together, these findings indicate that ACC neuronal populations, but not engrams, represent novelty, pain, and the dynamic valence of a CS. Our results provide new insights into how this brain region may play a role in discriminating the changing value of cues that predict threat.

Disclosures: P. Goswamee: None. **J.P. Burkett:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR091.11/II6

Topic: I.08. Learning and Memory

Support: RF1NS132913

Title: Geometry of mPFC Manifolds Predict Individual Differences in Skill Acquisition

Authors: *L. HE[1], Z. WANG[2], K. GANGULY[3];
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Abstract: Despite receiving equal training, individuals achieve different levels of motor skill acquisition across species and tasks. While most motor neuroscience studies focus on well-

trained subjects, little is known about what accounts for individual differences in long-term performance gain. Motor skill acquisition involves fast, cognitively driven improvements and slow, kinematic refinement, yet the neural mechanisms determining behavioral divergence remain unclear. Our recent work has suggested that the medial prefrontal cortex (mPFC) plays a role in the transition from early fast learning to the slower phase of late learning. Here, we investigate the role of the mPFC in shaping individual learning trajectories as well as determining how mPFC neural dynamics evolve over time. To test the potential causal relationship between mPFC and skill acquisition, we lesioned mPFC bilaterally prior to the learning. Interestingly, the mice were able to demonstrate performance improvements during the early phase (typically < 5 days), while showing no further long-term behavioral gains. We then monitored mPFC neural activity using a miniscope while the mice learned a cued reach-to-grasp task. We found evidence for the gradual emergence of sequential mPFC neural dynamics spanning long timescales, preceding the task initiation and persisting through the entire trial structure as well as covering the following intertrial period. This mPFC neural sequential dynamic could be embedded within a low-dimensional manifold, whose geometry reflected individual learning trajectory. Each animal's learning process resembled a search through a high-dimensional neural space toward a low-dimensional optimal subspace supporting consistent and accurate movements. This universal optimal manifold was shared among good-performing animals, while the individual deviation from the optimal geometry explained the behavioral differences across animals. Our results suggest that structured and stable mPFC is crucial for attaining better motor performance, whereas disorganized dynamics may lead to suboptimal skill levels. We interpret the emergence of such a universal mPFC neural manifold as important for 'understanding' the task goal and for monitoring the task outcome history, both are likely to be important for the long-term performance gain throughout skill acquisition. These findings highlight the cognitive contributions of mPFC to motor learning and provide a framework for understanding individual variability in skill acquisition.

Disclosures: L. He: None. **Z. Wang:** None. **K. Ganguly:** None.

Poster

PSTR091: Prefrontal Cortex Networks

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR091.12/II7

Topic: I.08. Learning and Memory

Support: STI 2030-Major Projects
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Title: Dynamic and persistent contextual state representations in orbitofrontal cortex

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Abstract: Adaptive behavior depends on the ability to track latent contextual states in order to apply appropriate rules. For example, humans adjust behavior based on the day of the week—working on weekdays, anticipating leisure on Fridays. While context can sometimes be externally signaled, it often must be internally maintained over extended periods without explicit cues and amidst distracting stimuli. This raises the question: how does the brain represent and sustain such internal contextual states? The orbitofrontal cortex (OFC) has been implicated in tasks requiring the representation of hidden or partially observable states. Lesions in the OFC impair performance in paradigms such as reversal learning, delayed alternation, and reinforcer devaluation, suggesting a deficit in distinguishing behaviorally distinct but perceptually similar contexts. However, the neuronal mechanisms by which the OFC encodes and maintains contextual states—while integrating ongoing sensory and task-related information—remain unclear. Do certain OFC neurons persistently encode contextual state independently of other task variables, or is this information multiplexed with other task variables in dynamic neural codes? To investigate these possibilities, we devised a task in which rats executed identical motor actions on each trial but received sucrose rewards only every third trial, amidst distracting odor cues. Like us tracking the passage of weekdays, rats were required to infer their proximity to future reward by maintaining an internal representation of a hidden contextual state. Single-unit recordings in OFC revealed that odor identity and contextual state were linearly dissociable at both single-unit and population levels. In contrast, individual neurons represented the current contextual state dynamically and nonlinearly integrated with information about ongoing task events. Critically, however, activity at the ensemble level could be used to reliably pull out the contextual states independent of lower-order task events. These findings offer crucial insights into how the brain integrates and dissociates higher-order context and lower-order task information to facilitate flexible learning and behavior.

Disclosures: F. Ma: None. **L. Zhang:** None. **J. Zhou:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Topic: I.08. Learning and Memory

Support: National Natural Science Foundation of China 32471082

Title: Action Outcome Mediates Neocortical Functional Plasticity during Sensorimotor Associative Learning

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Abstract: Learning to execute appropriate movements under different circumstances is critical for an individual's survival and well-being. Correctly converting input information to specific output movements relies on extensive cooperation among multiple regions in the neocortex. Nevertheless, how this intricate sensorimotor system is built throughout learning is not well understood. In this study, we recorded thousands of cortical neurons while mice learned to perform an olfaction-dependent delayed two alternative forced choice (d2AFC) task, which mice took on average 5.5 days to master. The learning process in most individual mice could be divided into three stages: Naive, Enlightening, and Expert. During the Naive stage, which typically lasted for about 3 days, mice were unaware of how to use odor cues to guide future choices. But after that, they gradually realized the association between sensation and action, and rapidly established a stable sensorimotor mapping. As learning progressed, neurons in multiple regions showed stabilized response patterns to sensory inputs and choices, and emerged to display selectivity for multiple task variables. The neurons with mixed selectivity increased the robustness of the cortical sensorimotor system, and their proportion accurately predicted the transition to the Enlightening stage at the behavioral level. Among cortical areas, the anterior-lateral motor cortex (ALM) became the critical hub for sensorimotor transformation in the Expert stage. In contrast, in the Enlightening stage, effective encoding of sensory and choice information involved widespread participation of cortical regions, including the posterior parietal cortex (PPC), the primary motor cortex (MOp) and even visual areas. Interestingly, ALM also received significant activation after mice made incorrect responses since they started to learn the task. Optogenetic inhibition of action-outcome-related signals in ALM prolonged the Naive stage. This outcome signaling was hypothesized to be coupled with the spiking activity of sensory- and choice-responsive neurons to facilitate credit assignment. In summary, our study reveals learning-induced plasticity in neocortical activity patterns, potentially linked to action-outcome information represented in ALM.

Disclosures: Y. Huang: None. **Q. Zhu:** None. **Z.V. Guo:** None.

Poster

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Topic: I.08. Learning and Memory

Support: AFOSR # FA9550-24-1-0325

Title: Dynamic and partially segregated neural representations in the Anterior Cingulate Cortex support complex task learning

Authors: *R. BULLICH VILARRUBIAS[1], E. ABS[2], R. BOEHRINGER[1], B. GREWE[1];
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Abstract: The ability to rapidly adapt to new behaviours and tasks is crucial for humans and animals living in complex, dynamic environments. Although this ability is a defining feature of mammalian intelligence, the mechanisms by which the brain uses existing knowledge to learn new tasks and integrate novel information remain largely unknown.The Anterior Cingulate Cortex (ACC) has been shown to play a crucial role in representing task schema, potentially facilitating the rapid learning of novel and increasingly complex tasks. We hypothesise that the ACC enables rapid learning by updating task-specific schemata through modular neural representations.To evaluate our hypothesis, we trained mice on a progressively complex task across multiple sessions while recording in vivo neural activity via calcium imaging in the ACC. Results show that behaviourally learned task features are encoded by distinct neuronal subpopulations, some exhibiting pure selectivity, while others display mixed selectivity to task features. As task complexity increases, neurons are recruited to represent the new task-relevant features while maintaining prior encodings. We used cosine similarity, linear decoders, and dynamical empirical modeling to investigate how neural representations generalize across sessions and task complexity, focusing on their structure and stability over time. Preliminary results suggest that task-related neural activity patterns reorganize in a structured manner during learning, reflecting a modular yet overlapping encoding scheme. While further analyses are needed, these findings point toward a potential mechanism by which the ACC supports flexible learning through dynamic and partially segregated population-level representations.

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Poster

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National Science Foundation

Title: Independent machine learning analyses identify prelimbic neural populations involved in safety learning

Authors: *K. SHULER, J. STEINHAUSER, E. PAPALEXAKIS, E. KORZUS;
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Abstract: The adaptive ability to properly distinguish between safe and threatening stimuli is fundamental to an organism's survival. The current project focuses on an area of the rodent medial prefrontal cortex, the prelimbic (PL) region, as it has previously been shown to modulate fear expression and disambiguate fear and safety cues. Mice were tested on a contextual fear discrimination learning paradigm with *in-vivo* calcium imaging to capture PL network dynamics across time and in different environmental contexts. Calcium imaging enabled the observation and quantification of neuronal network activity as the mouse learned to distinguish between neutral, safe, and threatening contexts during the paradigm. To identify latent factors that best represent our calcium data, we used two independent, unsupervised data mining techniques, Tensor Component Analysis (TCA) and graph-based community analysis. Both of these techniques uncovered three distinct subpopulations of neurons in PL that are highly co-active during specific behavioral epochs and contexts. While the consistency in activity patterns between the subpopulations defined by TCA and community analysis suggested that we had found an accurate depiction of PL network dynamics associated with contextual fear discrimination learning, we then aimed to verify if the same identified neurons in each TCA subpopulation were in fact members of the corresponding community. To do so, we compared the composition of each TCA- and community analysis-identified subpopulation and calculated the overlap of individual neurons. Thus, we were able to quantitatively confirm that the neurons that composed each of the TCA subpopulations also made up the majority of the corresponding community subpopulations with a high degree of overlap. In further characterizing the individual neuron makeup of each PL subpopulation, we validated that two independent computational approaches identified the same latent subpopulations within PL that were engaged during different phases of the behavior paradigm, indicating a strong case for the role of PL network dynamics in fear discrimination learning.

Disclosures: K. Shuler: None. **J. Steinhauser:** None. **E. Papalexakis:** None. **E. Korzus:** None.

Poster

PSTR091: Prefrontal Cortex Networks

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Program #/Poster #: PSTR091.16/II11

Topic: I.08. Learning and Memory

Support: NIH R00MH119320

Title: Parvalbumin interneurons show increased activity during safety learning in adolescence

Authors: *B. BLOCKER[1], R. A. SENNE[1], R. JAIN[1], C. LIN[1], H. C. MEYER[2];
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Abstract: Adolescence is marked by increased neural plasticity and dynamic connectivity changes that render this age group particularly sensitive to their environment. This period is also characterized by a heightened prevalence of fear-related diagnoses, with leading treatments, such

as cognitive behavioral therapies, being less effective. While there is evidence to suggest that safety learning paradigms are a promising approach to mitigate pathological fear in adolescents, the neural mechanisms underpinning safety learning are not fully understood. Previous work has shown that ventral hippocampal projections to the prelimbic (PL) prefrontal cortex have heightened activity during the presentation of safety tones, suggesting that an inhibitory circuit might be mediating the encoding of safety stimuli. To explore the role of parvalbumin interneurons (PVIs) in the presence of fear and safety stimuli, male and female adolescent and adult PV-Cre mice underwent four days of discriminative conditioning, during which mice were exposed to 20 second tones, consisting of 2 fear tones, terminated by a shock, and 28 safety tones. Both adults and adolescents were successful in discriminating by the fourth day. On the fifth day, the mice underwent a summation test, where 3 fear, safety, and compound (safety and fear tones played simultaneously) tones were presented, with fiber photometry recordings from PVIs in the PL. Across the board, adults and adolescents froze similarly during the summation test, with fear eliciting the highest freezing, safety eliciting the lowest, and compound in the middle. Fiber photometry data showed that safety presentations initially resulted in higher coordination of neural activity relative to fear presentations in all mice. However, adolescent PVI safety activity continued to increase throughout the cue, while adult PVI activity returned to baseline. During the fear cue, adult neural activity initially decreased, whereas adolescent activity was maintained at baseline throughout. Our results suggest that adolescent PVIs require both greater magnitude and duration of activation, potentially compensating for their decreased synaptic connectivity and overall prevalence in the PL to achieve inhibitory control comparable to adults. This work further elucidates the neural circuitry that underlie safety learning and guides the development of more effective treatments for adolescent fear disorders.

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Poster

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Topic: I.08. Learning and Memory

Support: The Jane Coffin Childs Postdoctoral Fellowship
Howard Hughes Medical Institute

Title: Prefrontal cortical neural dynamics underlying memory generalization

Authors: *X. SUN[1], S. HAZIZA[1], L. WANG[1], X. JIANG[1], O. HAZON[2], F. DINC[2], J. J. LI[1,4], Y. ZHANG[1,4], M. J. SCHNITZER[1,2,3,4];
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Abstract: Memories of daily events commonly evolve from more detailed to more abstract and generalized forms over time. This process, known as memory generalization, allows the gradual extraction of a memory's overarching features from its numerous specifics and may support or lay a basis for higher cognitive functions such as knowledge acquisition or problem-solving. However, the neural mechanisms by which generalization arises in the brain remain poorly understood. Here, we describe the emergence of prefrontal cortical neural ensembles and their dynamics that underlie the month-long generalization of memories formed during contextual fear conditioning.

Using activity-dependent genetic trapping, we identified a sparse population of neurons in the mouse prefrontal cortex that functionally matured during the generalization of contextual fear memorie*s*. Through bidirectional chemogenetic manipulations of these cells, we found that they were both necessary and sufficient to regulate generalization behaviors. Longitudinal neural calcium imaging studies revealed that these 'generalization cells' were activated during the initial conditioning sessions within a specific time window in the seconds after each footshock and only much later developed context-general activity patterns that triggered remote memory recall.

Neural population activity within this brief, post-shock interval window defined a low-dimensional subspace of the neural dynamics that was orthogonal to the dimension of context coding, and its emerging dynamics predicted generalization behaviors during remote memory recall. This reorganization in the dynamics of the prefrontal cortex was associated with a gradual strengthening of coordinated hippocampal-prefrontal dynamics involving generalization cells. A simple recurrent network model of hippocampal-prefrontal interactions recapitulated the temporal evolution of generalization cells. Altogether, these findings suggest that generalization arises from the active emergence of neural dynamics in the prefrontal cortex, providing a mechanistic framework for how the brain extracts general knowledge from specific experiences.

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Poster

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Topic: I.08. Learning and Memory

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Title: Changing neural representations of learned visual feature associations following acquisition of novel ones in primate dorsolateral prefrontal cortex during a virtual reality task

Authors: *B. MAHMOUDIAN[1], B. W. CORRIGAN[2], R. A. GULLI[3], A. J. SACHS[4], J. C. MARTINEZ-TRUJILLO[5];
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Abstract: To adapt to dynamic environments, animals must continuously integrate new information into previously established memories, requiring a balance between stability and plasticity of neural representations. However, it remains unknown how integrating new associations impacts old representations in the primate neocortex. To address this gap, we recorded from the dorsolateral prefrontal cortex (dLPFC; area 9/46d and 9/46v), a region causally involved in associative learning, as two macaque monkeys performed a familiar context-color association task both before and after learning of novel associations. Subjects navigated a virtual X-maze using a two-axis joystick while their eye movements were monitored. The wall texture of the maze corridor indicated the trial context (wood or steel). Upon reaching the corridor's end, the animals were presented with a colored object at the end of each arm of the maze (goal onset), with the trial context determining the rewarded object (wood-orange, steel-purple). Each day, the subjects performed a fixed context-color association (wood-orange, steel-purple) in the first and last blocks of the session. During the intervening blocks, the animals learned new context-color associations with a new set of colors daily in each novel block. Importantly, the context texture (wood or steel) remained the same throughout the session. This design allowed us to test for changes in neural responses to identical task features (e.g., context, color, target object) before and after learning of novel associations. Subjects showed stable performance for fixed (i.e., first and last) and novel association blocks. To evaluate single neuron selectivity to task features in fixed blocks (context, color, and target), neural activity during object foveations at goal onset was examined. We found that 35% of the neurons were selective to at least one task feature during foveations, with similar proportion of neurons selective across the fixed blocks for any given feature. Using a bootstrap procedure on a selectivity index (SI), we found that at least 20% of neurons selective for any given task feature lost or gained selectivity across the fixed blocks, suggesting a changing single neuron representation of task features within the sessions. This change was similar for neurons selective to the different features and was not attributable to the SI strength of a neuron. These results suggest that despite stable behaviour, neural representations of existing associative memories in dLPFC changes when new memories are incorporated. The latter highlights the dynamic nature of associative memory storage in the primate neocortex.

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Poster

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Title: Revisiting the Role of the Dorsolateral Prefrontal Cortex in Balancing Memory Systems: Evidence from Transcranial Magnetic Stimulation

Authors: *O. PESTHY[1,2], Z. PESTHY[3,4,2], T. VÉKONY[5], D. FABÓ[6,7,8,9], D. NEMETH[10,11,12]; [1]Ctr. de Recherche en Neurosciences de Lyon, INSERM, CNRS, Lyon, France, Bron, France; [2]Brain, Memory and Language Res. Group, Inst. of Cognitive Neurosci. and Psychology, HUN-REN Res. Ctr. for Natural Sci., Budapest, Hungary; [3]Doctoral Sch. of Psychology, [4]Inst. of Psychology, Eotvos Lorand Univ., Budapest, Hungary; [5]Gran Canaria Cognitive Res. Center, Dept. of Educ. and Psychology, Atlántico Medio Univ., Las Palmas of Gran Canaria, Spain; [6]Flash Clin., Budapest, Hungary; [7]Dept. of Neurol., Szeged Univ., Szeged, Hungary; [8]Dept. of Behavioral Sci., [9]Dept. of Voice, Speech and Swallowing, Semmelweis Univ., Budapest, Hungary; [10]CRNL - Ctr. de Recherche en Neurosciences de Lyon, INSERM, Bron, France; [11]BML-NAP Res. Group, Inst. of Psychology & Inst. of Cognitive Neurosci. and Psychology, Eötvös Loránd Univ. & HUN-REN Res. Ctr. for Natural Sci., Budapest, Hungary; [12]Gran Canaria Cognitive Res. Center, Dept. of Educ. and Psychology, Univ. of Atlántico Medio, Las Palmas of Gran Canaria, Spain

Abstract: Learning probability-based regularities, i.e., statistical learning, is fundamental for adaptive behaviour, skill acquisition, and habit formation. This process appears to be negatively associated with dorsolateral prefrontal cortex (DLPFC) functioning. It has been proposed that the DLPFC acts as a gatekeeper to long-term memory: its activation facilitates the retrieval of stored information, while its inhibition may allow for the acquisition of new probabilistic patterns. In this study, we tested this hypothesis by applying low-frequency (inhibitory) repetitive transcranial magnetic stimulation (rTMS) over the left, right, or bilateral DLPFC. We hypothesized that weakening the DLPFC would impair long-term episodic memory retrieval but enhance statistical learning. Additionally, we expected this effect to be driven primarily by left-hemisphere stimulation, based on hemispheric differences in cognitive strategies. Ninety-five participants were randomly assigned to one of four groups receiving sham stimulation or 1 Hz (inhibitory) rTMS over the left, right, or bilateral DLPFC. Participants completed a visuo-spatial probabilistic sequence learning task and a paired associate retrieval task in five times five minutes blocks. In the breaks between blocks, they received rTMS or sham stimulation. Results showed that the right and bilateral rTMS groups outperformed the sham group, with the right DLPFC group also exceeding the left DLPFC group. The stimulation had no significant impact on the episodic retrieval. These findings suggest that the DLPFC is more directly involved in modulating statistical learning than in balancing statistical and episodic memory processes. This challenges the theory that the DLPFC's primary role is to regulate access to long-term memory. Furthermore, contrary to our initial hypothesis, right rather than left DLPFC inhibition produced the strongest enhancement. This may reflect a shift toward broader spatial information sampling strategies resulting from reduced right DLPFC activity - beneficial for statistical learning, yet

without apparent consequences for episodic retrieval. Our findings contribute to a deeper understanding of the neural mechanisms underlying statistical learning.

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Poster

PSTR091: Prefrontal Cortex Networks

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Program #/Poster #: PSTR091.20/JJ1

Topic: I.08. Learning and Memory

Title: Prefrontal Cortex Activity During Golf Performance in Professional Golfers with Yips

Authors: *J. KIM[1], K. KIM[2], Y. W. YOON[3], Y. KIM[4];
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Abstract: The yips are a neurological phenomenon characterized by involuntary muscle contractions, tremors, focal dystonia, and impaired motor execution, which can negatively affect a golf swing. Therefore, the present study aimed to examine differences in motor cortex activity depending on task conditions. In this study, brain activity was measured using a wearable functional near-infrared spectroscopy (fNIRS) device (NIRSIT), which monitors changes in oxygenated hemoglobin in prefrontal areas including the dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), Ventrolateral Prefrontal cortex (VPC), and frontopolar cortex (FPC). Participants were professional golfers experiencing yips symptoms and performed two tasks while hitting a driver. In task 1, participants hit a driver without any target on the golf screen. In task 2, a circular target (20 meters in diameter) was added to the golf screen, allowing the participants to visually confirm whether their shot hit the target, thereby increasing performance pressure. After completing 10 driver shots for each task while wearing the NIRSIT device, the data were analyzed using a paired t-test to compare motor cortex activity between tasks 1 and 2. There was a **significant difference** in oxygenation levels, particularly in **DLPFC** (t =0.14, p = .045), **OFC** (t =0.81, p = .032). Also, oxygenation levels were **decreased in task 2** compared to task 1 in the **DLPFC** (Task 1 = 0.0026 ± 0.0004 vs Task 2 = -0.0092 ± 0.004), **FPC** (Task 1 = 0.0051 ± 0.0017 vs Task 2 = -0.0028 ± 0.0023), and **OFC** (Task 1 = 0.0021 ± 0.001 vs Task 2 = 0.0013±0.002). These results suggest that the presence of a target, which imposes high-pressure conditions, may reduce activation in prefrontal regions involved in motor control and cognitive regulation in golfers with yips. This reduction may reflect impaired cortical inhibition associated with performance anxiety. In light of these findings, future research will investigate the development of treatments based on neurophysiological mechanisms to improve yips-related motor dysfunction.

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Poster

PSTR091: Prefrontal Cortex Networks

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Topic: D.04. Brain Injury and Trauma

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HKRGC-GRF 14112523
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Title: The role of an ACC-BNST projection in sevoflurane-induced long-term cognitive impairment

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Abstract: The widespread use of general anaesthesia in paediatric surgery has raised growing concerns about its potential to cause enduring alterations in cognitive and memory function. Although this issue has been extensively investigated, the neural circuits involved in anaesthetic-induced memory impairments remain insufficiently defined. In this study, we focused on the anterior cingulate cortex (ACC) to bed nucleus of the stria terminalis (BNST) pathway, assessing its contribution to long-term fear memory deficits following repeated sevoflurane exposure. Male C57BL/6 mice were repeatedly exposed to sevoflurane for 3 days and subsequently subjected to Pavlovian fear conditioning and memory recall testing on day 21. Mice with prior sevoflurane exposure showed a significant reduction in freezing responses during long-term memory retrieval. This memory impairment was accompanied by altered c-fos expression in both the ACC and BNST. To further dissect neural dynamics during memory processing, real-time activity in the ACC and BNST were recorded using fiber photometry. In control animals, conditioned cues reliably evoked increased calcium activity in both regions. In contrast, this neural response was absent in mice previously exposed to sevoflurane. Notably, chemogenetic activation of the ACC-BNST circuit restored freezing behaviour in sevoflurane-exposed animals. Collectively, our findings suggest the ACC-BNST circuit as a critical substrate for sevoflurane-induced long-term memory deficits.

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Poster

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Title: Lateral frontoparietal functional connectivity based on individual sulcal morphology

Authors: *S. HÄKKINEN[1], W. I. VOORHIES[2], E. H. WILLBRAND[2,3], R.-H. TSAI[2,4], J. YAO[5], K. S. WEINER[2], S. A. BUNGE[2];
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Abstract: While cortical folding is a fundamental property of the brain linked with various cognitive skills, how sulcal morphology and functional brain architecture relate is less clear. Recent studies have emphasized putative tertiary sulci (pTS): small, shallow, late-developing, and evolutionarily new sulci, which have also been posited to serve as functional landmarks in association cortices. The present study follows up on a study in which the depths of three pTS in lateral prefrontal cortex (LPFC) were found to be strong predictors of reasoning performance in youth (Voorhies et al., 2021). Here, we characterized fMRI connectivity during reasoning task in participants drawn from the same sample (N = 43; 20 female; ages 7-18). Further, we tested whether the depths of these three pTS (*pimfs*, *pmfs-a*, *pmfs-i*) are associated with their connectivity. To do this, we assessed connectivity anchored to manually defined sulci in LPFC and lateral parietal cortex (LPC) (21 sulci/hemisphere, total of 1806). The functional relevance of sulci was then assessed using a combination of approaches: 1) Classification analyses demonstrated that sulci could be distinguished based on their functional connectivity patterns (mean pairwise accuracy 96%) and that their connectivity profiles were more discriminable than null models from spin testing (for the three pTS: $t_{164} = 34.69$; $p < 0.0001$). 2) Clustering revealed that the sulci nonetheless had groups with similar patterns. There was moderate agreement in cluster assignments at the group and individual levels, underscoring the need for individual-level analyses. Specifically, two exclusively pTS clusters emerged: one included 4/6 of the LPFC pTS previously involved with reasoning and the other connected pTS in LPFC and LPC. 3) Regression analyses demonstrated associations between sulcal depth and network centrality (degree, betweenness, participation coefficient) for various pTS, including several implicated with reasoning (left *pmfs-i,* right *pmfs-a*, left *pimfs*; $p < 0.05$, FDR-corrected). Additionally, we tested the necessity of laborious manual labeling by comparing connectivity fingerprints of manual and probabilistic sulcal definitions (>33% of participants). Four pTS did not have a probabilistic definition, and other sulcal fingerprints had an average correlation of 0.7 (ranging -0.51 to 0.98 across sulci and participants). In sum, the results highlight the functional relevance of fine-grained individual anatomy in frontoparietal cortex, such that sulcal-defined patches of

cortex share features of functional connectivity across participants and the depth of specific pTS associates with higher network centrality.

Disclosures: S. Häkkinen: None. **W.I. Voorhies:** None. **E.H. Willbrand:** None. **R. Tsai:** None. **J. Yao:** None. **K.S. Weiner:** None. **S.A. Bunge:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.01/JJ4

Topic: I.08. Learning and Memory

Title: Hippocampal-neocortical engram competition underlies preferential retrieval across multiple memories with different temporal scales

Authors: *Z. ZHAI, B. LEI, Y. ZHONG;
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Abstract: Living in dynamic environments, organisms require the ability to flexibly integrate prior knowledge with recent experiences. However, how animals integrate memories formed at different time points remains unclear. In this study, we developed a novel behavioral paradigm to investigate memory retrieval preferences between competing recent and remote memories in mice under different conditions. Our results revealed that female mice exhibited a stronger preference for retrieving remote memories compared to male mice, and such biased retrieval was mediated by increased activity in medial prefrontal cortex (mPFC) corticotropin-releasing hormone (CRH) neurons. Furthermore, the flexible retrieval between recent and remote memories could be expanded to other scenarios, including stress and social hierarchy change. These findings highlighted the competition between hippocampus-dependent recent memory and the neocortex-dependent remote memory at both behavioral and neuronal levels, and suggested that animals could adaptively modulate their multi-memory retrieval strategies based on internal states.

Disclosures: Z. Zhai: None. **B. Lei:** None. **Y. Zhong:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.02/JJ5

Topic: I.08. Learning and Memory

Support: NSERC CRSNG

Title: Success in transitive inference is associated with the activation of the medial prefrontal cortex in mice

Authors: *S. MARGARIAN[1], A. ALICANDRO[2], Y. CHEN[3], Y. YAO[2], T. HUI[4], K. TAKEHARA-NISHIUCHI[1];
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Abstract: Transitive inference (TI) allows us to predict indirect relationships between items and events based on previously learned direct comparisons. Past lesion studies have shown that TI relies on the integrity of the medial prefrontal cortex (mPFC) and hippocampus in both humans and rodents. However, the precise anatomical pathways underlying this essential component of intelligence remain poorly understood. By applying a gene-based circuit mapping approach to mice, we investigated brain regions whose activity correlates with successful transitive inference. We first developed a fully automated TI task in which items were five identical visual cues arranged horizontally on a wall (A-E). To prevent mice from mapping item relationships onto a linear spatial representation, the hierarchy among items was decoupled from an explicit spatial continuum (*i.e.,* the left side being consistently better than the right side). Male and female eight-week-old C57BL6/J mice were trained to discriminate overlapping premise pairs (A>B, B>C, C>D, D>E) where only the stimulus higher in the hierarchy was reinforced. After about one month of daily training, they underwent a recall test during which they were probed with a novel transitive pair (B vs. D) and a novel non-transitive pair (A vs. E) intermixed with trained premise pairs. During the recall test, ~80% of mice chose correctly in trained premise, novel transitive, and non-transitive pairs (high performers). In contrast, the rest chose correctly in trained premise and non-transitive pairs but not in transitive pairs (low performers). Analyses of cFos expression revealed that the prelimbic and infralimbic cortices of the mPFC were more strongly activated in the high performers than the low performers. In contrast, the CA3 subregion of the dorsal hippocampus showed similar cFos expression levels in both groups but significantly higher than in mice that remained in their home cage. Meanwhile, cFos expression in the dentate gyrus and CA1 subregion did not vary based on task performance or test participation. Currently, we are examining cFos expression in additional brain regions as well as the overlaps between cFos expression and efferent projection targets. These results suggest that the strong mPFC activation is a key predictor of successful transitive inference.

Disclosures: S. Margarian: None. **A. Alicandro:** None. **Y. Chen:** None. **Y. Yao:** None. **T. Hui:** None. **K. Takehara-Nishiuchi:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.03/JJ6

Topic: I.08. Learning and Memory

Support: Alzheimer Society of Canada Proof of Concept Grant
CFI Leaders Opportunity Fund
Data Sciences Institute Doctoral Student Fellowship

Title: In silico modeling of amyloidosis-induced CA1 spike abnormalities: Unraveling the role of excess synaptic glutamate

Authors: *Y. SUN[1], J. TAXIDIS[3], K. TAKEHARA-NISHIUCHI[2];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive accumulation of beta-amyloid, leading to a marked decline in episodic memory. AD-related amyloidosis is known to disrupt synaptic transmission and plasticity and induce network hyperexcitability in the hippocampus and neocortex. Through in vivo recordings from the dorsal CA1 of the TgCRND8 mouse model of amyloidosis, we found that memory deficits are associated with disrupted experience-driven changes in spike dynamics during sharp wave ripples (SPW-Rs) and impaired restructuring of functional coupling among excitatory cells. To investigate the circuit-level dysfunction responsible for these abnormalities, we used an established cortical-CA3-CA1 computational model that generates physiologically accurate SPW-Rs and spike dynamics in morphologically simplified CA1 and CA3 pyramidal neurons and interneurons. Experience-dependent changes in SPW-R LFP and spike dynamics, consistent with experimental data, emerged from the model following the strengthening of synapses within a subset of CA3 pyramidal neurons and their projections to CA1 pyramidal neurons. We then simulated two dysfunctions commonly observed in AD patients and mouse models: excess synaptic glutamate and interneuron dysfunction, both implicated in neuronal hyperexcitability. Excess synaptic glutamate was modeled by enhancing excitatory synaptic transmission from CA3 pyramidal neurons to all CA1 neurons. This manipulation reproduced all observed abnormalities in the TgCRND8 mouse model, including reduced baseline SPW-R modulation of firing, weakened experience-driven increase in firing, and a global increase in coactivity but no change in multi-neuron coactivity structure. In contrast, interneuron dysfunction, modeled by reducing inhibitory synaptic transmission within the CA1, only replicated some of these findings but produced opposite effects on basal SPW-R modulation and multi-neuron coactivity. Overall, these results support the view that excess synaptic glutamate is a key circuit-level dysfunction linking beta-amyloid to disruptions in experience-triggered CA1 network modifications, processes essential for memory encoding.

Disclosures: Y. Sun: None. **J. Taxidis:** None. **K. Takehara-Nishiuchi:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.04/JJ7

Topic: I.08. Learning and Memory

Support: Alzheimer's Society of Canada
CFI Leaders Opportunity Fund
Data Science Institute

Title: Amyloidosis disrupts experience-driven coactivity formation among hippocampal CA1 neurons and impairs memory encoding

Authors: Y. SUN[1], S. CHEKHOV[2], S. MARGARIAN[2], P. BOGLE[3,2], C. HAN[3], P. E. FRASER[4,5,6], ***K. TAKEHARA-NISHIUCHI**[2,1,6];
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Abstract: Deficits in episodic memory are a cardinal symptom of Alzheimer's disease (AD). Uncovering the physiological and circuit abnormalities underlying these impairments is crucial for linking AD molecular pathology to cognitive dysfunction. Transient bursts of oscillatory activity known as sharp wave ripples (SPW-R, 100-250 Hz) occur throughout the hippocampus and are necessary for the consolidation of episodic memory. Although several studies using mouse models of AD reveal abnormalities in SPW-Rs, it remains unclear how spike dynamics during SPW-Rs supporting memory consolidation are affected. Using *in vivo* electrophysiology, we recorded neuronal activity from the dorsal CA1 hippocampus of 3- to 5-month-old TgCRND8 (Tg) mice with AD-related amyloidosis and their healthy littermates (non-Tg). Recordings were performed during rest periods before (PRE) and after (POST) mice were subjected to an object-location recognition memory task. We found that many CA1 excitatory cells from both Tg and non-Tg mice increased their firing rates during SPW-Rs prior to task exposure. During POST-task periods, this SPW-R modulation of firing rate was increased in a subset of non-Tg excitatory cells. In contrast, most excitatory cells from Tg mice did not show the same level of experience-dependent increase. These experience-sensitive cells in non-Tg mice also developed a higher probability of joint activity among them, but not with other excitatory cells, indicating the formation of cell assemblies encoding the experience. This specific refinement of functional coupling between experience-sensitive cells was abolished in Tg mice, which could not recognize object displacement after 3 hours. Finally, the formation of new multi-neuron coactivity structures was observed exclusively in non-Tg mice, whereas non-Tg mice exhibited only an overall increase in coactivity. Together, these results demonstrate that AD-related amyloidosis disrupts experience-dependent reorganization of coactivity among CA1 excitatory cells, thereby impairing a process essential for memory formation.

Disclosures: Y. Sun: None. **S. Chekhov:** None. **S. Margarian:** None. **P. Bogle:** None. **C. Han:** None. **P.E. Fraser:** None. **K. Takehara-Nishiuchi:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Program #/Poster #: PSTR092.05/JJ8

Topic: I.08. Learning and Memory

Support: McKnight Brain Research Foundation
UF CAM Center
UF Center for Advanced Spatial Biomolecule Research

Title: The effects of aging, diet, and APOE genotype on the brain lipidome

Authors: *H. STROTHER[1], D. ZAMBRANO RIOS[1], H. LI[1], C. SOTO[1], R. RIBAS[1], A. P. MAURER[2], J. L. BIZON[4], R. SUN[1], S. N. BURKE[3];
[2]Evelyn F. McKnight Brain Inst., [3]Neurosci., [1]Univ. of Florida, Gainesville, FL; [4]Neurosci., Univ. of Florida Dept. of Neurosci., Gainesville, FL

Abstract: Lipid metabolism is critical for normal brain function, and lipidome dysregulation is emerging as a defining feature of aging and age-related cognitive disorders. Interestingly, the greatest genetic risk factor for late onset Alzheimer's disease (LOAD) is the E4 variant of the *APOE* gene, which encodes a lipid transporter. This highlights the necessity of determining how aging and genetic risk for LOAD impact lipid metabolism. Spatial metabolomics with matrix assisted laser desorption/ionization (MALDI) mass spectrometry allows for unprecedented insight into how aging and *APOE* genotype alters the brain lipidome with spatial resolution <50µm. This study aimed to characterize the spatial lipidome in young (7 months) and aged (23 months) rats and how region-specific lipid enrichment was altered by 3 months of a high-fat, low-carbohydrate 'ketogenic' diet. Furthermore, to understand how this interacts with *APOE* genotype, humanized knock-in rats with selective replacement of the WT *APOE* gene with the human E3 and E4 variants were examined for their physiological response to ketosis-inducing treatment. These rats were also behaviorally tested on the novel object recognition task. There were dramatic differences between the lipid profiles of young and aged rats across the brain, that showed distinct alterations in different brain regions. A ketogenic diet increased lipid enrichment throughout the hippocampus and appeared to lessen the age differences within specific hippocampal subregions. Interestingly, the peripheral response to ketosis-inducing treatments differed by *APOE* genotype and sex even though there were no significant genotype or sex effects on object recognition. To further interrogate the effect of *APOE* genotype on cognition and the brain lipidome, future experiments will employ additional cognitive tests and conduct MALDI imaging on the brain tissue of *APOE* rats to examine the interactions between cognitive aging, genotype, and lipid composition.

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Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Program #/Poster #: PSTR092.06/JJ9

Topic: I.08. Learning and Memory

Support: The McKnight Brain Research Foundation
UF CAM Center
NIH/NIA grant R01AG049722 to SNB

Title: Object Discrimination Deficits in Aging: Dissecting Perirhinal Cortex Function Using DREADDs and fMRI Approaches

Authors: *A. ROSS[1], R. BALAJI[2], Z. ERDOGAN[2], A. P. MAURER[3], S. N. BURKE[1], S. A. JOHNSON[4];
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Abstract: Cognitive decline in aging significantly impairs the ability to distinguish between similar stimuli, affecting both everyday decision-making and memory-guided behavior (Reagh et al., 2014; Reagh et al., 2018; Berron et al., 2021). This discrimination deficit has been linked to dysfunction in the cortical-hippocampal circuit, including the perirhinal cortex (PRC), a region critical for resolving feature ambiguity during object recognition and stimulus discrimination (Burke et al., 2011; Johnson et al., 2017). Age-related changes in PRC function (Burke et al., 2014; Burke et al., 2018), including evidence of reduced neural activation during cognitive tasks, may compromise its role in perceptual and mnemonic discrimination. Building on evidence that age-related hyperactivity and excitatory/inhibitory imbalance in the cortical-hippocampal circuit contribute to cognitive deficits (Yassa et al., 2011; Maurer et al., 2017), this study aims to determine whether these deficits are exacerbated by chemogenetic inhibition of the perirhinal cortex (PRC). The current study utilized Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to transiently inhibit PRC activity in young (4 months) and aged (22 months) Fischer 344 x Brown Norway F1 rats during an object-based mnemonic similarity task (MST). In this task, rats must discriminate between object pairs consisting of a trained target (S+) and lure (S−) objects that vary in their visual similarity (0-90% feature overlap). To achieve selective and reversible inhibition in the PRC, we used an inhibitory viral construct (pAAV-hSyn-hM4D(Gi)-mCherry (AAV2)) in combination with the DREADD actuator deschloroclozapine (DCZ, 1 mg/kg, i.p.). PRC inactivation impaired discrimination performance. Initial data also indicate that aged rats exhibit baseline deficits on the task, consistent with prior evidence of age-related discrimination abilities. Future work will determine whether enhancing excitatory drive in the PRC using a stimulatory DREADD (pAAV-hSyn-hM3D(Gq)-mCherry (AAV2)) modulates object discrimination and alters large-scale functional connectivity as measured by resting-state fMRI. These complementary manipulations aim to clarify the functional contribution of PRC activity to mnemonic discrimination and circuit-level alterations in aging, providing a foundation for future interventions targeting discrimination deficits in age-related cognitive decline.

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Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Topic: I.08. Learning and Memory

Support: McKnight Brain Research Foundation
UF CAM Center
NIH/NIA R01AG049722 to SNB
F31AG082445 to SS

Title: The Role of Sex and Age on Hippocampal- Striatal Interactions During Spatial Navigation and Associative Learning

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Abstract: Aging in spatial navigation tasks is associated with a shift from hippocampal (HPC)-dependent spatial strategies to dorsal striatum (DS)-driven response strategies (Barnes 1980; Pereira et al. 2015; Colon-Perez et al. 2019). However, it remains unclear whether this shift also extends to associative learning tasks in which age animals show a bias towards response driven behavior that interferes with task acquisition. To explore this, we tested young and aged male and female Fischer 344 x Brown Norway F1 hybrid rats in a land-based and water-based T-maze, along with an object paired-place association task (OPPA; Lee & Kim, 2010; Jo & Lee 2010) that requires rats to learn which of two objects is the correct choice in different spatial locations of a two-arm maze. As observed in earlier studies (Gardner et al., 2020; Tomas-Pereira et al., 2015), aged males favored DS-dependent strategies in the water-based maze compared to younger males. Females, regardless of age, consistently employed response-based strategies. Furthermore, spatial strategies were similarly maintained across land and water-based mazes for all rats; however, their response-driven biases were not reflected in the OPPA task despite the well-documented vulnerability of the hippocampus in aging (Rogers 2012; Gardner et al. 2020). This suggests that cognitive aging reflects task-specific changes, rather than a global shift towards a DS-dependent behavior. Future work will include the analysis of individual behavioral strategies of our rodents for both spatial and associative modalities. We will also compare 2D and 3D immunohistology from each brain to evaluate the activity patterns in the hippocampus and dorsal striatum during response-based behavior.

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Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Program #/Poster #: PSTR092.08/JJ11

Topic: I.08. Learning and Memory

Support: R01 AG072714

Title: Age-related changes in intraneuronal glucose availability in medial prefrontal cortex during working memory performance

Authors: *S. ZEQUEIRA[1], M. FARAJI[2], Z. KRUMM[3], S. N. BURKE[3], B. SETLOW[4], J. L. BIZON[5];
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Abstract: The brain is highly energy consuming, and metabolic changes in neurons are essential for neural plasticity and cognitive function. The primary energy source for the brain and neural activity is glucose and aging can be associated with brain glucose hypometabolism, impaired transport of essential substrates underlying metabolism, and mitochondrial dysfunction. Such impaired brain energy availability may contribute to cognitive deficits associated with aging and age-related neurodegenerative disease. Prior work evaluating brain glucose metabolism have largely relied on methods such as positron emission tomography, autoradiography, magnetic resonance spectroscopy, and microdialysis, but these methods lack the spatial and/or temporal resolution necessary to assess glucose dynamics in real time during behavior. Recent advances in fluorescent methodology now allow for visualization of glucose dynamics at sub-second timescales in awake, behaving animals. Specifically, iGlucoSnfr is a recently developed genetically encoded biosensor that utilizes the glucose-sensing domain from a bacterial protein fused to a fluorescent reporter. We used the iGlucoSnfr glucose sensor to evaluate the contributions of brain glucose availability to working memory performance across the lifespan. Young adult (5 months) and aged (23 months) Fischer 344 x Brown Norway F1 hybrid rats were food-restricted and pre-trained on a medial prefrontal cortex (mPFC)-dependent delayed response working memory task. The delayed response working memory task required rats to remember a previously shown location of a lever over variable delay periods ranging from 0-18 s. Rats then underwent surgical injection of pAAV.hSynap.(cyto)iGlucoSnfr-mRuby2 into the mPFC, followed by implantation of an optic fiber into the same site. Rats were tested on the delayed response task while undergoing simultaneous photometric recording of glucose availability in mPFC via a fiber optic tether. Photometry signals were collected via an RZ10x system and Synapse software (Tucker-Davis Technologies). The $\Delta F/F$ signals were aligned to significant task events (e.g., correct vs. incorrect choices), z-scored relative to baseline periods during inter-trial intervals. Data to date show notable alterations in glucose availability during the delay periods of the task, during which rats have to maintain information in working memory, before returning to baseline following trial completion. These data suggest that glucose availability in the mPFC varies as a function of cognitive load.

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Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Program #/Poster #: PSTR092.09/JJ12

Topic: I.08. Learning and Memory

Support: R01MH126236

Title: Quantifying hippocampal-prefrontal circuit engagement using LFP and surface EEG in the Paired Associates Learning task

Authors: *C. BESOSA[1], Y. QIN[1], A. KEIL[2], S. N. BURKE[1], A. P. MAURER[1];
[1]McKnight Brain Institute, Dept. of Neurosci., Univ. of Florida, Gainesville, FL; [2]Dept. of Psychology, Univ. Florida, Gainesville, FL

Abstract: Disrupted hippocampus (HPC)-prefrontal cortex (PFC) coordination is a hallmark of mental health disorders, yet the mechanisms linking local cellular interactions to large-scale neural signals across behavioral states remain unclear. This project aimed to determine whether surface EEG could be used to infer HPC-PFC LFP coordination with skull electroencephalogram (EEG) recordings that could be used to develop physiological biomarkers for testing the efficacy of therapeutics to treat mental illness. We accomplished this by analyzing the direct link between local field potential (LFP) recordings from the HPC and PFC and EEG during rest, running on a circle maze, and the Paired Associates Learning (PAL) task. The PAL task, which evaluates visuospatial associative memory, requires animals to learn image-location pairings in an operant based touchscreen apparatus. Prior work shows that disruptions in HPC-PFC coordination—such as those caused by traumatic brain injury or advanced age—impair PAL performance. Similarly, human studies have linked EEG dynamics during PAL with behavioral deficits in schizophrenia and anxiety. Rats were trained on the PAL task and then implanted with 64-channel silicon probes as well as skull EEG recording sites. Our findings reveal increased theta-band frequency and power correlations in HPC and PFC during PAL comparable to running but higher than rest. Our results also indicate that theta-band dynamics exhibited the most pronounced behavioral modulation across brain regions, with interregional correlations primarily restricted to low frequencies. Power and phase relationships between LFP and EEG suggest aggregate HPC synaptic activity is predictable from EEG parameters, so we are developing a computational model aimed at reconstructing local field potentials from noninvasive surface EEG data. Ongoing experiments are pairing electrophysiology recordings with nucleus reuniens (RE) inactivation, since this thalamic area is reciprocally connected to both HPC and PFC, and we previously showed significant decreased PAL performance following RE inactivation. Exploring the relationship between invasive LFP and surface EEG signals across levels of performance in a translatable touchscreen paradigm could provide new physiological and behavioral biomarkers that will aid the development of therapies for mental health disorders. Funding R01MH126236.

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Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Program #/Poster #: PSTR092.10/JJ13

Topic: I.08. Learning and Memory

Support: The McKnight Brain Research Foundation
UF Center for Cognitive Aging and Memory
The McKnight Brain Institute Accelerator Award to APM

Title: Dynamics of intraneuronal glucose levels in female APOE3 and E4 rats during behavior and insulin tolerance testing

Authors: *J. JUERGENSMEYER[1], S. D. LOVETT[2], A. P. MAURER[2], S. N. BURKE[2];
[2]Evelyn F. McKnight Brain Inst., [1]Univ. of Florida, Gainesville, FL

Abstract: Disrupted glucose utilization is a ubiquitous feature of brain aging and often precedes pathological signatures of age-related neurodegenerative disease. Ketogenic diets—which shift metabolite usage from glucose to ketone bodies—have shown promise in mitigating these deficits. Furthermore, the E4 variant of the apolipoprotein E (*APOE*) gene, which is the greatest genetic risk for late onset Alzheimer's disease, is also associated with disrupted glucose metabolism prior to old age, highlighting the critical importance of understanding neuro-metabolism across the lifespan. However, to date, no study has examined the dynamics of neuronal glucose utilization across different metabolic states in association with glycolysis versus ketosis. The present study measured intraneuronal glucose dynamics in the hippocampus of female homozygous *APOE3* and *E4* knock-in rats under both ketogenic and control diets in relation to behavior as well as insulin tolerance testing. Adult (4-7 months) female *APOE3* and *APOE4* humanized knock-in rats were trained to perform a continuous spatial alternation task, injected with pAAV.hSynap.(cyto).iGlucoSnFR2.mRuby3 in the hippocampus to express an intraneuronal glucose sensor, and implanted with a fiber optic cannula for in vivo photometry. Each rat alternated between ketogenic (high fat, low carbohydrate) and control diets. Blood glucose and β-hydroxybutyrate levels were measured at the start and end of each session to confirm metabolic state. In addition, rats received intraperitoneal injections of insulin (0.75 U/kg). Insulin injections resulted in notable changes; first, a rise in fluorescence as glucose uptake increased, followed by a decline. During a ketogenic diet, glucose levels changed more rapidly compared to a control diet condition. These findings establish that fiber photometry of glucose levels can be used to monitor hippocampal neuronal glucose dynamics in relation to behavior and insulin responses, and that a ketogenic diet improves neuro-metabolic flexibility, as evidenced by faster changes in glucose levels in response to insulin. Future work will expand these observations by comparing neuronal glucose dynamics between E4 and E3 rats as well as correlating glucose transients with task performance.

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Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

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Program #/Poster #: PSTR092.11/JJ14

Topic: I.08. Learning and Memory

Support: SECIHTY-CBF2023-2024-1641
P3C2025-UDG

Title: Prediabetic state impairs recognition memory in a sex-specific pattern

Authors: *D. E. SILVA SILVERIO[1], S. J. LOPEZ-PEREZ[2];
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Abstract: Type 2 diabetes mellitus (T2DM) is a major risk factor for cognitive decline and dementia. Increasing evidence suggests that cognitive alterations may begin during the prediabetic stage. Middle age represents a critical period for identifying early cognitive and metabolic dysfunctions. In this study, we aimed to determine whether a high-caloric diet (HCD) induced prediabetic state is associated with recognition and spatial memory impairments in male and female mice during middle age. CD-1 mice of both sexes, aged 3 months and weighing approximately 30 g, were fed either a standard diet (control) or an HCD starting at 3 months of age. After 24 weeks of dietary intervention, at 9 months of age corresponding to approximately 40-50 human years metabolic and cognitive assessments were conducted. Body weight and fasting glucose were monitored regularly. Glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed to confirm a prediabetic phenotype; data were analyzed using area under the curve (AUC) calculations. Recognition memory was evaluated using the Novel Object Recognition (NOR) test with a 24-hour retention interval to assess long-term memory. Spatial memory was assessed through the Barnes radial maze test. Statistical analyses were performed to assess the effects of diet and sex on metabolic and cognitive outcomes. Our preliminary findings show that HCD-fed mice developed features consistent with a prediabetic state, including impaired glucose and insulin tolerance, confirmed by AUC analyses of GTT and ITT. Importantly, both male and female mice in the HCD group exhibited significant deficits in recognition memory compared to controls ($p = 0.044$ for males, $p = 0.007$ for females). However, no significant differences were observed in spatial memory performance, suggesting that the cognitive impact of the prediabetic state may be selective to recognition memory. Notably, correlation analyses revealed a weak, non-significant negative association between GTT AUC and discrimination index in males ($r = -0.017$, $p = 0.967$). In contrast, female mice displayed a significant negative correlation ($r = -0.812$, $p = 0.049$), suggesting that greater

glucose intolerance was associated with poorer recognition memory performance. These results indicate that prediabetic conditions can negatively impact cognitive function during middle age, with possible sex-specific differences in vulnerability.

Disclosures: D.E. Silva Silverio: None. **S.J. Lopez-Perez:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.12/JJ15

Topic: I.08. Learning and Memory

Support: NSFC/RGC/JRS (N_HKU735/21)
RGC/GRF (17102120)
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HMRF (09200966)

Title: The Influence of Targeted Memory Reactivation During Sleep on Fear-Induced Memories and the Mechanisms Underlying

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Abstract: Targeted memory reactivation (TMR) is a non-invasive technique to manipulate memory consolidation during sleep after learning. Studies have shown that TMR during the non-slow-wave sleep (NS) period of non-rapid eye movement sleep can impair the consolidation of fear memory, but the mechanism underlying this remains unclear. Sleep spindle is a waxing and waning electroencephalography rhythm between 10-14 Hz. As it mainly occurs during NS and is closely related to memory consolidation, the principle of TMR influencing memory might be disclosed by focusing on this sleep component. In this study, we tried to apply TMR to the sleep spindle after the fear conditioning and measure the effect of TMR on memory with the freezing behaviour in the fear recall test. A closed-loop system, which is threshold-based, was utilised to perform the online sleep staging, spindle identification, and deliver the TMR stimulus during the sleep recording. Customised models were also developed to identify the freezing behaviour of the mouse based on DeepLabCut training. Based on current data, we found no significant difference in the fear memory between mice that received spindle-TMR one day after fear learning and controls, which aligns with the previous finding that 24h-TMR during NS did not affect fear memory. By understanding the effect of TMR during sleep, this study can investigate

how memory consolidation is influenced during sleep and potentially inform new approaches for memory-related intervention.

Disclosures: L. Jin: None. **Q. Zheng:** None. **C.S. Lai:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.13/JJ16

Topic: H.02. Reward and Appetitive Learning and Memory

Support: Gdansk University of Technology, IDUB grant: 1/1/2024/IDUB/I.1B/Pt
National Science Centre, Polonez Bis: 2022/47/P/NZ4/03358

Title: The Switchboard Test: A flexible, low-cost setup for assessing spatial memory in rats during sequential instrumental tasks

Authors: *R. SAHU[1], W. ZAKOWSKI[2], K. MACKIEWICZ[2,1], O. POLNIAK[2], D. WISNIEWSKI[3], J. KOTUS[3], J. NIEDIEK[4], J. AGGLETON[5], S. O'MARA[6], M. M. JANKOWSKI[1];
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Abstract: Appetitive instrumental conditioning is widely used to shape animals' associations between actions and outcomes under controlled conditions. When implemented in automated environments with more complex tasks, it enables the collection of high-resolution data across hundreds of trials, supporting fine-grained behavioral analysis. Our work introduces an automated, low-cost interactive environment for studying spatial memory in rats. The system enables training and testing of location- and sequence-specific responses, with adjustable parameters including spatial layout, sequence length, cues, timing, and reward size. It is an open-field environment (64 × 64 cm) featuring a single reward dispensing area and nine equally spaced floor buttons (3 × 3 arrangement) that rats can press using a fraction of their body weight. The system operates using an Arduino controller and a PC to manage floor switches, a reward dispenser, an amplifier with a speaker for playback of feedback sounds, and a 64-LED array that signals reward delivery. It is designed for compatibility with electrophysiology and deep brain stimulation (DBS), providing a flexible tool for behavioral studies. Over several weeks, rats (n = 16) were gradually trained to memorize a target button-press sequence (7→4→2), which outlined a fixed spatial route within the arena. This sequence was selected as a moderate-

difficulty option based on a free-choice control protocol where rats chose among all four possible extensions of the 7→4 sequence (7→4→1, 2, 5, 8). Each trial was self-initiated and, if successfully completed, ended with a distinct sound (dolphin vocalization) and a green light cue, indicating reward delivery and signaling successful trial completion. Errors resulted in a distinct feedback sound and immediate termination of the trial. Upon activation, each switch in the target sequence triggered a unique sound from a set of complex natural sounds, such as those of crickets, small frogs, and dolphins, intended for use in future experiments to test the transfer of spatial memory to the abstract auditory domain. In expert rats, removing all feedback sounds had a minimal effect. Based on experimental data, we proposed a memory score calculation to generate learning curves, offering a sensitive metric of spatial memory performance. This study presents a novel approach to evaluating spatial memory within a flexible, dynamic operant conditioning framework.

Disclosures: R. Sahu: None. **W. Zakowski:** None. **K. Mackiewicz:** None. **O. Polniak:** None. **D. Wisniewski:** None. **J. Kotus:** None. **J. Niediek:** None. **J. aggleton:** None. **S. O'Mara:** None. **M.M. Jankowski:** None.

Poster

PSTR092: Learning and Memory: Cellular, Network, and Behavioral Studies

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR092.14/JJ17

Topic: I.08. Learning and Memory

Support: NSF Grant IOS-1942202
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Title: Differences in general, dopaminergic, and serotonergic activity between personality types while learning in zebrafish (Danio rerio)

Authors: *J. M. CORCORAN[1], R. Y. WONG[2];
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Abstract: Individual differences in learning can contribute to differential survival success. One factor that contributes to individual differences is an animal's personality type. Studies generally demonstrate that bold individuals tend to learn faster than shy individuals. However, the underlying neural mechanisms are not well understood. Personality type and learning performance are both separately associated with modulation of the dopaminergic and serotonergic signalling systems in the brain. Therefore, these systems may explain the link between personality type and learning speed. In this study we test the hypothesis that differences in learning performance between personality types are mediated by specific neural activity patterns (e.g, general, dopaminergic, and serotonergic) across the brain. Using immunohistochemistry, we quantified neural activity in the medial zone of the dorsal

telencephalon, lateral zone of the dorsal telencephalon, ventral hypothalamus, pre optic area, subpallium, pretectum, habenula, superior raphe, pars posterior of area dorsalis, and posterior tuberculum in animals undergoing associative learning with a food reward after eating food, 3 days of conditioning, and after 7 days of conditioning. After 3 days of conditioning there were no differences in time spent in the conditioned zone between control and treatment or bold and shy fish. Investigations into neural activity patterns suggest a complex interaction between learning and personality type. These results help us understand the mechanisms of learning differences between personality type and may also help to explain cognitive biases between individuals.

Disclosures: J.M. Corcoran: None. **R.Y. Wong:** None.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR093.01/JJ18

Topic: I.08. Learning and Memory

Support: NIH grant R01MH137669

Title: A neural frame of reference

Authors: *L. A. AREND, E. PARK, A. A. FENTON;
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Abstract: Complex distributed processes require temporal coordination between interacting subcomponents. Certain complex systems, such as microchips, jazz bands and neural ensembles, generate their own process-centric axis of time along which events within the system are ordered. We show that the hippocampus has normal modes of resonance spanning 3-30 Hz, with maximum phase coherence at 7 Hz theta oscillations. A normal mode is an extracellular field state in which all parts of the system oscillate at the same frequency but with fixed phase offsets. The normal mode creates a standing wave along the dorsal-ventral axis of CA1 and DG of the hippocampal formation. The standing wave has nodes and antinodes which are anchored to anatomical features and universally conserved across subjects and across time within the subject. We treat the nodes as invariant neural landmarks and use them to spatially register 9 two-hour acute Neuropixels recordings made in 5 mice. We treat cycles of the 7 Hz normal mode as clock ticks uniformly spaced along a time axis generated by hippocampal computation. Neural time flows uniformly not with respect to clocks in the lab but with respect to cycles in the brain. Putting the two notions of space and time together we define a neural frame of reference, a physical coordinate system in which both distance and duration are defined purely with respect to neural markers. This frame of reference is an absolute metric space in which measurements of neural geometry can be meaningfully compared across time, sessions, and subjects. We assume the perspective of an observer in the neural frame, and report a number of metrics commonly used to describe brain function in order to assess how neural measurements transform under this

neurocentric projection. We describe how LFP coupling, neuronal cofiring, and population vector embeddings change when viewed in the neural frame, instead of the laboratory frame. The neural frame of reference turns out to be privileged in that when spikes are viewed within it, statistical laws that explain how neural events relate to space and time become identifiably simpler. Adopting this perspective makes it possible to rigorously study and explain aspects of cognition without making reference to stimulus or behavior.

Disclosures: L.A. Arend: None. **E. Park:** None. **A.A. Fenton:** None.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

Location: SDCC Halls B-H

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Topic: I.08. Learning and Memory

Support: Chan Zuckerberg Initiative (CP2-1-0000000614)

Title: Identifying a memory manifold in the hippocampal transcriptome using linear/non-linear gene-gene covariance and Louvain clustering

Authors: *J. HAN[1], A. B. GRAU-PERALES[2], R. M. HARRIS[4], H.-Y. KAO[1], A. PAL[1], J. M. ALARCON[5], T. C. SACKTOR[6], S. MARTINIANI[1], H. A. HOFMANN[7], A. A. FENTON[3]; [2]Dept. of Neurosci., [3]Ctr. for Neural Sci., [1]New York Univ., New York, NY; [4]Lake Tahoe Community Col., South Lake Tahoe, CA; [5]Pathology, SUNY Downstate Hlth. Sci. Univ., New York, NY; [6]Dept Physiol. & Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [7]Integrative Biol., The Univ. of Texas at Austin, Austin, TX

Abstract: The hippocampus is an essential part of the brain, responsible for encoding long-term memories through complex molecular processes. However, conventional transcriptome analyses struggle to link the gene expression changes to known protein-level observations. For example, despite being crucial for maintaining wildtype LTP and long-term memory, Protein Kinase Mζ RNA(*Prkcz*), shows no significant change after active place-avoidance memory training in standard bulk RNA-seq DEG results, even though the PKMζ protein increase two-fold for at least a month.

The shortcomings of focusing on single gene associations to behavioral variables like memory motivated us to adopt a population-level gene-network approach to determine if the organization of gene-gene covariance relationships in the transcriptome could detect PKMζ and related memory-related changes. One day after training, we profiled transcriptomes from the hippocampal tri-synaptic circuit—dentate gyrus (DG), CA3, and CA1—in active place avoidance-trained and control mice that had the identical physical experience but did not form an avoidance memory. We then developed a three-tiered analysis framework: (1) DEG analysis to capture genes with large differential expression between conditions, which identified immediate early genes (IEGs) such as *Fos*, *Fosl2*, *Arc*, and *Npas4*; (2) Combined correlation metrics of the

non-linear Spearman and xi correlations against a multivariate behavioral principal component measure of memory (PC^{memory}). The goal was to detect both monotonic and complex gene-behavior associations, expanding the candidate gene set to include transcripts with subtle but behaviorally relevant covariation; (3) Iterative Louvain clustering on a reconstructed gene-gene network, generating probabilistic co-clustering profiles that score genes by relational covariance, revealing network hubs whose community membership shifts between conditions.
While DEG analysis alone highlights only IEGs, our integrated pipeline also identified key genes *Prkcz* and *Wwc1*(KIBRA) of the PKMζ-KIBRA protein complex crucial for memory maintenance. Along with IEGs these genes are primary anchors of the control-to-trained transition in gene expression relationships, revealing molecules whose functional importance at the protein level is "in the shadow" of conventional transcriptome analyses. This population-level network-driven strategy thus reveals both apparent gene expression changes and subtler regulatory modules, as we begin to uncover the hidden molecular architecture of hippocampal memory formation and retention.

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Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Program #/Poster #: PSTR093.03/JJ20

Topic: I.08. Learning and Memory

Support: NIMH NRSA F32MH135680
NIMH Grant R01MH132204

Title: Coordinated cognitive control signals in dorsal hippocampal and anterior cingulate representations during navigation

Authors: *G. J. BLAIR[1], A. A. FENTON[2];
[1]Ctr. for Neural Sci., NYU, New York, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: We aim to understand how information is dynamically represented across distinct brain regions. Navigating our daily lives requires the judicious use of limited cognitive resources to yield preferred outcomes, called cognitive control, which is known in humans and animals to depend on the medial prefrontal cortex (mPFC), including the anterior cingulate cortex (ACC). Our studies of rats and mice performing an active place avoidance task have also demonstrated the importance of hippocampus (HPC) for cognitive control. We performed simultaneous imaging using miniscopes in the ACC and HPC of freely-behaving rats to determine how information is separately represented in these two areas. Rats were transfected with AAV9-

CaMKII-GCaMP8m in HPC dorsal CA1 and ACC, then gradient refractive index lenses were implanted above the injection sites. Rats were trained to perform an Active Allocentric Place Avoidance (AAPA) task or an appetitive Spatial Memory and Allocentric Reinforcement Training (SMART). We recently developed the SMART task as an appetitive complement to AAPA, to further evaluate cognitive control in an appetitive decision making framework. Both tasks are conducted on a rotating circular arena, which dissociates the local arena cues from the stationary distal room cues. Rats must suppress the local distractor cues while using the distal room cues to guide either avoidance (in AAPA) or appetitive navigation (SMART). Because room and arena cues continuously change their relationships, both tasks require cognitive control to utilize the appropriate task-relevant cues. Ensemble recordings of ACC and of CA1 demonstrate that cells in both regions are significantly modulated by position, though CA1 activity is more location-specific (greater mutual information). Analysis of the momentary positional information (I_{pos}) time series computed from simultaneously recorded CA1 and ACC ensembles revealed a highly correlated spatial frame-specific representation of current location that purposefully alternates between the arena and room frames signaling cognitive control in both tasks. Importantly, this information is spatially coordinated in a task relevant manner, with more room information in the shock vicinity (AAPA) or at the decision junction (SMART task). Decoding position from each region reveals that the two representations become more correlated upon approach to the shock zone, which develops with task acquisition. These coordinated cognitive control signals in rodent ACC and CA1 enable investigations of a potential mechanism of how two distinct brain circuits coordinate the information needed to guide behavior.

Disclosures: G.J. Blair: None. **A.A. Fenton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BioSignal Group.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR093.04/JJ21

Topic: I.08. Learning and Memory

Support: 1R56MH133929-01 Neurobiological Basis of Reference Frame Coordination for Spatial Memory to Action Transformations 25-992341-WSQPG-S5238

Title: A sparse random network coupled to a continuous attractor network explains the transformation of spatial representations during head-fixation

Authors: *J. R. HURTADO[1], A. A. FENTON[2];
[1]New York Univ., New York City, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Spatial navigation relies on computations distributed across many brain regions, including the hippocampus, entorhinal cortex, and thalamus. Place cells in the hippocampus encode position, while head direction cells in the anterior thalamus encode head direction. Head direction signals are known to rely on specialized circuit connections (i.e. attractor networks), while specialized connectomes seem less relevant for hippocampal place cells. Furthermore, it is not clear how the brain combines distinct network types to generate a coherent sense of space across a vast array of possible inputs. Disrupting vestibular input—as observed in head-fixed mice—alters the geometry of population activity in the thalamus while preserving its circular topology. These transformations highlight the dependence of representational structure on specific input streams. At the same time, effective navigation depends on two complementary processes: path integration, which updates internal estimates of position using self-motion cues, and landmark anchoring, which uses external cues to recalibrate these estimates. A unified account must explain both representational changes under sensory disruption and the integration of internal and external cues. We propose a hybrid model combining two network types: (1) a DivSparse network—an unstructured, sparsely connected circuit with inhibitory feedback that receives multi-modal external inputs and produces sparse, localized tuning; and (2) a continuous attractor network (CAN) with structured recurrent connectivity that maintains a stable network state. The DivSparse output anchors to sensory cues and the CAN can integrate velocity signals, while neither model can do both on its own. This work explores a simple example where structured internal dynamics interact with unstructured architectures while adapting to external cues, reproducing several empirical phenomenon. When combined, the network corrects internal estimates generated in the attractor, while stabilizing activity when cues are unavailable. This architecture, termed the pATTERN model (place Attractor EI Random Network), can explain the transformations in the population activity of neurons in head-fixed mice and also make predictions about the mechanisms coupling internal and external senses of space.

Disclosures: J.R. Hurtado: None. **A.A. Fenton:** None.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Topic: I.08. Learning and Memory

Support: NIH Grant R01MH132204-01A1

Title: How to search for the Engram: Interpreting the results of Optogenetic Engram manipulations.

Authors: *C. GARCIA JOU[1], D. CRUZ-HOLDEN[2], E. PARK[4], S. CARRILLO SEGURA[3], A. A. FENTON[4];
[1]City Univ. of New York, New York City, NY; [3]Ctr. for Neural Sci., [2]NYU Ctr. For Neural Sci., New York, NY; [4]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Optogenetics has revolutionized our understanding of memory formation, storage, and retrieval by enabling the precise manipulation of a subset of memory-tagged neurons that results in mice expressing the conditioned behavior. Because the stimulation is sufficient for expressing the memory, the memory-tagged cells have been called “engram cells.” However precise, this use of optogenetics is still the manipulation of a complex system with adaptive, non-linear, and homeostatic interactions. Interpreting these experiments to be causal manipulations has been confusing because precisely-timed neurophysiological patterns of neuronal network activity are thought to generate memory-associated behaviors. Because the network response to the optostimulation is unknown, we investigated effects of optogenetic stimulation of memory-tagged cells in hippocampal CA1 ensembles. We used Arc-CreERT2-ChR2 mice to study the effects of optostimulation during anesthesia, head fixation, and foraging in a linear track. Some “tagged” cells (18% of total), reliably responded to the light pulses (15-ms @ 4 or 10 Hz) with sub-second latency. Optostimulating under urethane anesthesia increased single-unit discharge and cofiring. Prolonged (10 min) optostimulation under head-fixation and freely-moving conditions resulted in a progressive reduction of evoked responses in tagged cells and other cells with elevated activity. The CA1 network rapidly adapts with cells increasing, decreasing, or maintaining their baseline activity, regardless of their classification as tagged or non-tagged. During optostimulation, CA1 also maintains cofiring relationships and thus the low-dimensional manifold organization of the population dynamics. In the foraging task, CA1 place cells were identified by numerical criteria. Ten-minute baseline and optostimulation trials had comparable proportions and quality of place cells (36% vs 27%, p’s>0.17). Contrary to expectations that tagged cells simply respond to stimulation, the firing fields of tagged cells were more stable than non-tagged cells. Overall CA1 was resistant to change under optostimulation. This correctly predicted that the stimulation was sufficient to elicit a conditioned active place avoidance memory in a neutral environment. We conclude that optogenetic manipulations of memory-tagged cells are correctly interpreted as eliciting the endogenous population dynamics of a complex system, rather than a demonstration that the tagged cells are the substrate of memory.

Disclosures: C. Garcia Jou: None. **D. Cruz-Holden:** None. **E. Park:** None. **S. Carrillo Segura:** None. **A.A. Fenton:** None.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Program #/Poster #: PSTR093.06/KK1

Topic: I.08. Learning and Memory

Support: NIH grant R01NS105472
Simons Collaboration for the Global Brain (SCGB), DC004260

Title: On the sense of direction with fixed vestibular sensations

Authors: *A. A. FENTON[1], J. HURTADO[2], S. CARRILLO SEGURA[4], A. PAK[5], D. E. ANGELAKI[3];
[1]New York Univ., New York, NY; [2]Ctr. For Neural Sci., New York Univ., New York City, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY; [4]Ctr. for Neural Sci., NYU Ctr. For Neural Sci., New York, NY; [5]Ctr. for Neural Sci., Nazarbayev Univ., Astana, Kazakhstan

Abstract: Azimuth is encoded by the activity of head-direction cells (HDCs) providing an allocentric sense of direction. While it has been natural to think of these cells as responding to environmental features that set allocentric direction, it is also clear that the sense of allocentric direction and the activity of HDCs is neurocentric, internally-organized, driven also by egocentric senses like vestibular stimulation and self-movement. Allocentric and neurocentric direction appear to be synchronized during free locomotion but are in discord in the virtual reality setups that are now popular. Here we investigated the contribution of vestibular stimulation to HDC network activity by investigating HDCs during active navigation without changing vestibular sensations. We recorded anterior-dorsal nucleus of the thalamus HDCs while mice freely explored either a stable environment or a floating Neurotar environment during head fixation. Exploration of the Neurotar environment allowed self-motion so the mice could explore the normal visual, olfactory, auditory and tactile features of an environment, but without changing vestibular sensations. HDCs lose allocentric directionality during head-fixed locomotion but preserve HDC cofiring that defines a neurocentric ring manifold that unstably registers to the environment. “Velocity gain” is the ratio between the neurocentric and allocentric angular velocities, explicitly coupling neurocentric direction to azimuth. A unitary velocity gain enables an accurate allocentric direction sense, but velocity gain changes episodically, depending on behavior and sensory features of the environment. When the head-fixed mice were stationary, HDC activity appeared to be off the ring manifold, but this was because the intact manifold shifted in the HDC-state space to an orientation that was nearly orthogonal to the orientation during active behavior. In fact, these manifold shifts were transiently observed in the freely-behaving mouse, especially during darkness. These results indicate that, in the absence of vestibular cues, the HDC ring manifold dynamics persist but do not reliably register to external environmental features. We find that the organization of the HDC ring manifold in the absence of vestibular stimulation is explained by a “subjective drift” model where the coupling to the environment shifts episodically and subjectively to different environmental features, while maintaining its internal organization. These findings point to why orienting is subjective, challenging in VR setups, and why vestibular-sensory conflicts can disrupt memory and what we know.

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Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Topic: I.08. Learning and Memory

Support: R01MH132204-01A1

Title: A novel tool for manipulating plasticity in memory tagged neurons

Authors: *A. GARCIA[1], D. M. CRUZ-HOLDEN[2], A. A. FENTON[2];
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Abstract: Synaptic plasticity is the ability of synapses to change their strength or efficiency in response to activity or experience. According to the synaptic plasticity and memory (SPM) hypothesis, the maintenance of synaptic strength is necessary and sufficient for memory storage (Martin et al. 2000). PKMζ has been shown in wildtype conditions to be the only known protein required for the maintenance of late-phase LTP and long-term memory (Pavlowsky et al. 2017). This makes PKMζ an ideal candidate for critically testing the SPM hypothesis. So far, there does not exist a reliable strategy to manipulate synaptic plasticity in memory-related neurons. However, recent advances in genetic tools have enabled genetic manipulations in memory-related neurons. Targeted Recombination in Active Populations (TRAP) is a method that labels transiently active neurons. Using this approach, we've shown that photo stimulation of the memory tagged neurons in a neutral environment is sufficient to elicit a learned avoidance behavior (Jou et al. 2023). This occurred despite only tagging 18% of the neurons (in CA1), suggesting that this approach recruits the relevant memory-related neurons. However, it is unclear if this was independent of the maintenance of synaptic plasticity in these neurons. Here, we utilize TRAP mice crossed with Prkcζ floxed (Prkcζfl) and (STOPfl) ChR2-eYFP mice to selectively eliminate Prkcζ in active neurons during recall. Using immunohistological samples, we measured the expression of PKMζ in the aforementioned experimental mice and compared it to control mice that did not have Prkcζ floxed. Specifically, we compared the raw integrated density (RID) of PKMζ in areas with high eYFP expression to areas with low eYFP expression as the areas with high eYFP expression are the putative manipulated areas. Our immunohistochemical results indicate that the raw integrated density (RID) of PKMζ is lower in areas with high eYFP expression compared to areas with low eYFP in experimental animals, However, this ratio was equal in control mice. This suggests that our manipulation was effective at eliminating the expression of PKMζ in memory tagged neurons. Given that PKMζ is crucial for maintaining wild type LTP, this observation suggests that this strategy may be able to specifically manipulate synaptic plasticity in memory-tagged cells (eYFP+). In this case, this strategy is ideally suited to critically investigate the SPM hypothesis by directly testing if the maintenance of activity induced synaptic changes in memory tagged neurons is necessary for long term memory.

Disclosures: A. Garcia: None. **D.M. Cruz-Holden:** None. **A.A. Fenton:** None.

Poster

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Title: Remote and predictive coding during dentate gyrus immobility theta provides a mechanism for mental exploration during working memory

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Abstract: Mental exploration enables flexible evaluation of future choices without requiring real-world iterations and facilitates the execution of working memory (WM), especially when many items need to be remembered. When increasing the memory load in simple spatial WM tasks, they become dependent on the dentate gyrus (DG) though the neural mechanisms remain elusive. We have shown that the DG is essential for prospective coding in the downstream CA3 recurrent network during dentate-dependent spatial WM. Compressed neural activity patterns within hippocampal sharp wave ripples (SWRs) that play out future paths in advance of behavior were proposed as a candidate mechanism. However, real-time disruption of awake SWRs did not impair memory performance or disrupt upcoming decisions in our dentate-dependent WM task. In search of other types of neural activity that support mental exploration during WM, we uncovered a novel hippocampal mechanism specialized for predictive coding. Unexpectedly, prominent theta oscillations (4-12 Hz) were observed in the rat dorsal DG during periods of immobility in the dentate-dependent WM task, challenging the conventional view that rodent hippocampal theta is either movement-dependent or sensory-evoked. The perceived prominence of movement theta was a result of previous studies primarily assessing the oscillations and neural activity of the CA1 hippocampal subregion during behavior. By using Neuropixels probes (n = 8 rats), we simultaneously assessed all hippocampal subregions and compared the local field potential, current source densities and neuronal spike activity during behavior. We observed that theta oscillations were undetectable at CA1 recording sites during movement pauses, as commonly reported, but at the same time, bouts of prominent high-amplitude theta were observed in the DG. During DG immobility theta, slow gamma-phase coupling was decreased in CA1 but increased within the DG-CA3 network, suggesting stronger intrahippocampal CA3-DG computations than during movement theta. By analyzing hippocampal population activity (n = 921 cells) during immobility theta, we revealed structured neural activity patterns (“theta sequences”) that represented multiple remote choice paths, particularly the next behavioral choice. In contrast, movement-related theta sequences primarily code for the animal’s immediate surroundings. By demonstrating remote and predictive coding in hippocampal neural activity during DG immobility theta, our findings provide a new conceptual framework for understanding the neural mechanisms in support of mental exploration.

Disclosures: M. Wang: None. **Y. Li:** None. **Y. Zhang:** None. **P. Ee:** None. **J. zhu:** None. **S. Leutgeb:** None. **J.K. Leutgeb:** None.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Title: Neural Sequences in Striatum Encode Future Behavioral Choices

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Abstract: The neural mechanisms by which long-term working memory supports the execution of future intentions remain incompletely understood. Prospective coding—when neural population activity reflects future behavioral outcomes prior to execution—has been proposed as a candidate substrate for working memory. Prior studies have identified such prospective coding in neural population activity within regions involved in spatial working memory, including the hippocampus, medial entorhinal cortex (mEC), and medial prefrontal cortex (mPFC). However, it remains unclear how memory-related signals from these upstream areas are transformed into motor plans within downstream circuits. Here, we investigated whether the dorsal medial striatum (DMS), a major output target of mEC and mPFC, exhibits prospective coding—in the form of temporally structured population activity that predicts future actions—during a delay-based spatial task in which optimal performance required an alternation between right-turns and left-turns at a T junction. Using Neuropixels recordings in DMS (n = 4 rats, 5 sessions per rat, 403±98 neurons per session) and applying seqNMF to extract neural sequences, we identified a temporally structured sequence that reliably emerged at the exit of the delay zone and thus before an overt behavioral choice was executed. Although single-neuron activation selectivity within the sequence was weak and overall activity appeared similar across left-turn and right-turn trial types, this sequence carried sufficient information for a linear decoder to predict the animal's upcoming turn direction with over 80% accuracy. This dissociation suggests that subtle, distributed differences in DMS neural activity are integrated at the population level to represent upcoming actions based on memory-related input. In contrast, a distinct sequence observed on the return arm—prior to the delay—yielded lower decoding accuracy (~60%), underscoring that striatal neural sequences may be specific for memory retrieval and/or converting stored memory into a committed motor plan immediately before action execution, but not for retrospective

memory encoding. These findings support a model in which DMS contributes not only to action execution but also to working memory retrieval and/or action planning. By integrating subtle differences in activation strength across a subset of cells within temporally structured population codes, the striatum may serve as a downstream integrator for memory-driven motor planning. This function extends classical models of working memory retention beyond hippocampal-cortical circuits.

Disclosures: J. Wu: None. **X. Li:** None. **J. Wang:** None. **Y. Li:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Title: Circuit-level insights into dopaminergic modulation of movement in parkinsonian models

Authors: *S. GONZALEZ[1], Z. XIAO[1], S. KOOIMAN[1], S. HARLALKA[1], Y. LI[1,2], B. LIM[1], J. K. LEUTGEB[1,3], S. LEUTGEB[1,3];
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Abstract: The basal ganglia (BG) play a central role in action selection and motor control through two major output pathways: the direct and indirect pathways, classically thought to have opposing effects on movement. Recent evidence suggests these pathways operate in a coordinated manner, modulated by dopamine (DA), to govern movement initiation and cessation. Here, we examine models of BG function by investigating how progressive DA loss alters striatal dynamics by recording large-scale activity using Neuropixels probes in the dorsomedial and dorsolateral striatum of mice performing a virtual cued goal task. We compared controls to MitoPark mice at 8 weeks (MitoPark, no DA loss or motor impairments: n = 8; Control: n = 10) and 12 weeks (MitoPark, moderate DA loss and early motor impairments, n = 6; Control n = 3). Although 12-week-old MitoPark mice maintained behavioral accuracy, they exhibited impaired movement initiation, mirroring early Parkinson's symptoms when initiation difficulties precede broader decline. Single-unit analyses (average number of cells/session = 184, 27 sessions in 17 mice) revealed that firing in response to reward delivery (reward ON) declined progressively with age and DA depletion, while decreased firing (reward OFF) did not emerge, suggesting a

selective loss of excitatory reward signaling rather than a polarity shift. Notably, changes in striatal reward coding appeared before measurable DA loss, implicating early functional disruption. By 12 weeks, when significant DA fiber loss was evident, reward ON responses declined further, indicating progressive DA loss exacerbates reward-related deficits. In contrast, increased and decreased firing during movement stops (stop ON and stop OFF responses) showed distinct dynamics: during maturation from 8 to 12 weeks, stop ON responses declined and stop OFF responses increased, while MitoPark mice showed a trend towards early increases in both, followed by diminished responses with advanced DA depletion. These results suggest DA depletion destabilizes the balance of reward and stop signaling, leading to a reduction in task-related activity. Our findings highlight that DA loss disrupts the temporal coordination of BG pathways beyond simple firing rate changes, refining our understanding of BG circuit dysfunction in Parkinson's disease and informing strategies for circuit-targeted therapies.

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Poster

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Walter F. Heiligenberg Professorship
Kavli Institute for Brain and Mind
JSPS International Fellowship

Title: Periodic and memory-related firing patterns of neurons in dorsal medial entorhinal cortex span retention intervals in working memory tasks

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Abstract: Memory retention over tens of seconds is critical for working memory (WM), yet the neural mechanisms that maintain information over extended retention intervals remain incompletely understood. Sequential firing of cells in the hippocampus (HPC) and medial entorhinal cortex (MEC) has been suggested as a mechanism for memory retention during delay intervals in spatial WM tasks. Yet, our prior work has not observed sequential hippocampal firing beyond a few seconds during delay periods, irrespective of whether theta oscillations during the delay were continuous or intermittent. To investigate whether information might be stored in MEC over longer time intervals than in HPC, we used Neuropixels probes to record

dorsal and ventral MEC neurons in a spatial WM task with 30-second delays (n = 8 rats). In contrast to the time-limited firing patterns in HPC, we observed a variety of firing patterns in MEC that spanned the entire delay interval. First, a substantial proportion of neurons coded for either the upcoming or past choice (dorsal MEC, with/without running on a treadmill during the 30-s delay: 14.7%/17.1%, n = 2635 cells; ventral MEC: 13.3%/11.9%, n = 963 cells). Second, with running during the delay, a substantial proportion of dorsal MEC cells (23.8%, 627 cells) fired periodically aligned to the distance traveled on the treadmill rather than the elapsed time. Among these distance-periodic neurons, 41.7% were classified as grid cells when recorded in an open field following the WM task. Third, the distance-periodic cells organized into repeating sequences over the course of treadmill running, forming internally generated firing patterns that repeated as the animal ran on the treadmill with the head in a stationary position—indicating internally computed distance metrics. Intriguingly, the same sequences reappeared in forward order after the delay and in backward order before the delay, showing that dorsal MEC ensembles can dynamically reverse or maintain sequence direction based on task phase. In dorsal MEC without running on the treadmill and in ventral MEC in any condition, neurons exhibited transient temporally ordered sequential activity at the onset of the delay, lasting ~5 s, followed by a steady state of persistent firing. Therefore, the distance-periodic coding and sequence organization was only observed in dorsal MEC with treadmill running, while memory-coding was consistently observed in dorsal and ventral MEC. These results highlight a unique role of dorsal MEC in generating internal metrics of distance and in organizing firing patterns through running and/or theta-dependent mechanisms, distinct from HPC and ventral MEC contributions to WM.

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Poster

PSTR093: Neural Mechanisms Underlying Memory and Spatial Navigation

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Simula/UiO/UCSD PhD Program (SUURPh)

Title: Modulation of Hippocampal Spike Timing by Septal GABAergic Inputs

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Abstract: Hippocampal theta oscillations are thought to emerge from coordinated interactions between local excitatory networks and long-range inhibitory input from the medial septal area (MSA). The two oscillators are coordinated such that the pace of hippocampal excitatory cells is slightly faster than the inhibition-driven network that generates the local field potential (LFP), which results in phase precession (PP) of excitatory cells with reference to LFP theta. PP is thought to be of high relevance for hippocampus-dependent behavior because it results in compression of behavioral scale sequential activity (e.g., time cells) to theta-scale sequential activity (i.e., theta sequences) within the time range of synaptic plasticity mechanisms. While two oscillators have thus been implicated in sustaining theta rhythms and generating PP, their relative dominance and functional interplay remains unclear. Here, we investigated whether hippocampal principal cells can maintain PP and temporal coordination when the slower rhythm is externally manipulated by pacing GABAergic MSA cells, which are known to disinhibit entorhinal and hippocampal interneurons. We expressed ChR2 in MSA and applied frequency-specific optogenetic stimulation to interleaving blocks of trials while mice (n = 5) performed a spatial working memory task. During the task, we recorded hippocampal LFP and CA1 neurons and examined the neurons' spike-phase relationship. We found that phase locking strength was strongly modulated by stimulation frequency—12 Hz and 20 Hz stimulation induced robust locking to the artificial rhythm, often exceeding endogenous theta locking. However, PP with reference to the endogenous theta frequency remained largely intact across all conditions, even when only a minor remaining signal was detected at the endogenous frequency under strong septal entrainment to the artificial frequency. Conversely, PP relative to the artificial stimulation frequency was weak or absent, with only a limited dependence of spike-phase dynamics on pacing efficiency. The resilience of PP at the endogenous frequency suggests that hippocampal temporal coding is not solely dependent on the LFP reference signal generated by septal pacing. Rather, the finding that PP persists under rhythmic interference without being overridden by external long-range rhythmic drive points to a robust mechanism of theta coordination that integrates local inputs. Therefore, a second oscillator—governed by local and/or entorhinal networks rather than by septal entrainment—may control PP and behaviorally relevant temporal firing patterns in the hippocampal network.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Topic: I.03. Decision Making

Support: NIDA Grant 5R21DA056241-02

Title: Understanding the roles of single dopamine neurons in feeding motivation in Drosophila

Authors: *P. M. BLACK;
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Abstract: Understanding the neural basis of internal states is a focus of neuroscience research. Dysregulated internal states are a hallmark of many psychiatric illnesses, yet we are only beginning to uncover how internal states are generated, maintained, and utilized to guide behavior. Research using *Drosophila melanogaster* to investigate internal state has been held back by available assays. In particular, the typical assay for feeding motivation, bitterness conflict, is confounded by starvation. As a consequence of this, how flies generate changes in their decision making in conditions of deprivation is not well understood. To address this, we developed a novel assay with a non-confounded barrier to feeding, food hardness, via agarose. We validated our novel assay using three basic motivators, finding that flies became more willing to work for protein, sugar, and salt when they had faced prior specific deprivations. After validating our assay, we then set out to find populations of neurons that could be responsible for the internal states created by nutrient-specific motivations. We found that a broad population of dopamine neurons were required for protein drive, yet inessential for sugar or salt drives, a comparison which had, to our knowledge, never been carried out. To ensure rigor, we replicated every experiment on multiple days, with a total n of between 45 and 60 (135-180 animals). After finding a broad neuronal population with a specific role in internal state, we then turned to the recently released *Drosophila* Brain Simulation in order to generate single-neuron candidates that could provide the circuitry needed to drive the motivated feeding we observed. The simulation revealed a subpopulation of central brain dopamine neurons that could change the flies behavior in our assay conditions. Importantly, highly specific split-GAL4 driver lines were available for a number of our candidates found using the simulation. We are in the process of testing these dopaminergic neurons using thermogenetic tools to determine how the fly brain changes itself to create the internal state necessary to seek out an essential nutrient.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Topic: I.03. Decision Making

Support: NSF GRFP 2237827
MnDRIVE DSI Fellowship
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Title: Parameter-specific effects of electrical stimulation on cognitive control

Authors: *E. M. DASTIN-VAN RIJN[1], M. BUCCINI[1], J. BENNEK[1], E. M. SACHSE[2], A. S. WIDGE[3];
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Abstract: Deep brain stimulation (DBS) is a promising therapy for treatment-resistant psychiatric disorders. Recent work in humans and rodents suggests that the therapeutic effect of DBS arises from enhancing cognitive control-the ability to flexibly adjust decision making in the face of distractors or changing context. Traditionally, cognitive control is measured via decreases in reaction times on relevant behavioral tasks. These measures could be leveraged by an optimization platform to objectively assess the potential therapeutic efficacy of stimulation parameters in a personalized manner. To determine the parameter-dependence of these stimulation related-changes, Long-Evans rats (n=15) were trained on an extradimensional set-shifting task. In this task, rats had to shift between selecting stimuli based on side or light to receive rewards. This task requires cognitive control in two ways: suppression of a prepotent response to the light in favor of the currently rewarding option and switching strategies to the presently rewarding stimulus type. Rats were implanted with a single stimulating electrode in the right hemisphere mid-striatum. Rats completed two types of task schedules of five sessions each: interleaved blocks of stimulation at varying amplitude and frequency or optimizing sessions where amplitude was selected to continuously minimize reaction times according to a Gaussian process model. Interleaved blocks of active stimulation showed a comparable decrease in reaction time (β;=-0.077, $p<0.0001$) to prior studies also without decreasing accuracy ($p>0.05$) demonstrating that the stimulation related enhancements in cognitive control have a rapid onset and can be measured acutely. This acute improvement was only present for amplitudes greater than 100 μ;A ($p<0.0001$) for both 130 Hz and 20 Hz stimulation. In the optimizing sessions, all rats showed locations in amplitude-space where reaction times were minimized. However, the location and magnitude of these effects varied substantially between animals. Together these results demonstrate that stimulation-related improvements in cognitive control can be rapidly measured and are sensitive to the choice of parameters supporting the feasibility of a future personalized stimulation platform.

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Poster

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Title: Neurotest: an open-source tool for characterizing and assessing electrophysiology equipment

Authors: *J. MORROW[1], H. CHO[3], Y. TIWARI[2], T. DENISON[5], J. A. HERRON[4], A. S. WIDGE[2];
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Abstract: Electrophysiological recordings of neural activity are sensitive to noise from a variety of sources including electronics that are either integrated into the experimental setup (e.g., a pulse generator) or are present in the adjacent environment (e.g., power supplies). Disentangling electrical artifacts from biological signals can be challenging given the lack of knowledge about the underlying ground truth of data acquired from a live subject. This issue is particularly pernicious in closed-loop paradigms, where neural events need to be tracked and responded to in real time. The NeuroTest Board (NTB) was designed to help address this issue by providing researchers with a tool to both assess and test equipment in a controlled environment. The easy to assemble device consists of an arbitrary current source waveform generator and a data acquisition system featuring configurable filters and gain settings. Control software operated via the open-source Arduino IDE is easy to use and can be customized to fit the individual needs of experimenters.
Here, we outline a series of simple tests that allow electrophysiologists to characterize and assess how components of their setups function. We start by showing that the NTB is well calibrated to ensure microvolt-level precision for both the data acquisition and signal generation. The calibrated system is then used to demonstrate how integrating external amplifiers (e.g., an intan RHD2132 chip) or signal generators (e.g., Keysight 33500B and StimJim) impact the noise floor of in vitro recordings. Next, we show how to use the signal generator of the NTB to assess the fidelity of data acquisition (DAQ) systems. This is done by comparing a known output from the NTB injected into a saline tank against the signal recorded on the DAQ. We demonstrate this functionality with both programmed waveforms and by replaying neural data recorded from the rodent brain into the saline tank. Lastly, we show how the NTB can be used to simulate evoked response potentials (ERPs) in vitro and demonstrate how a commonly used amplifier, the intan RHD2132, attenuates ERPs present in the first few milliseconds following certain stimulation pulses.
These tests demonstrate the value of the NTB as a benchtop tool for researchers within the broader electrophysiology and closed-loop neuromodulation fields.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

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National Institute of Neurological Disorders and Stroke of the National Institutes of Health 1R01NS120851-01A1

Title: Exploring Behavioral Flexibility Through Deep Brain Stimulation: Insights from a Two-Armed Spatial Bandit Task in Rats

Authors: *I. WAKU[1], E. M. DASTIN-VAN RIJN[2], E. M. SACHSE[3], N. GLEWWE[4], E. RENN[5], J. BENNEK[5], M. BUCCINI[5], A. E. REIMER[5], B. A. EBITZ[8], N. M. GRISSOM[6], A. R. DE OLIVEIRA[9], A. S. WIDGE[7];
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Abstract: Cognitive flexibility - the ability to make flexible, goal-oriented decisions - is often impaired in disorders such as Major Depressive Disorder and Obsessive-Compulsive Disorder, which involve cortico-striatal dysfunction and exhibit sex differences in prevalence and treatment response. Our group previously showed that mid-striatal DBS (homologous to human Ventral Capsule/Ventral Striatum) enhances flexibility by reducing reaction time (RT) in a rat set-shifting task. Its effects on decision-making under uncertainty, including explore-exploit dynamics, remain unknown. The two-armed spatial bandit task probes these processes, and prior studies revealed sex-specific strategies in mice. To investigate circuits underlying behavioral flexibility and sex differences, we adapted a two-armed spatial bandit task for rats (n=4M/4F). Next, rats were implanted with stimulating electrodes targeting the mid-striatum. Stimulation parameters were: ON (300μA, 50μs, 130Hz), pseudo-randomized trial-based (PR), or OFF. We used Hidden Markov Models to infer latent exploratory and exploitative states, and quantified RT, probability of exploration (p[explore]), win-stay/lose-shift strategies, and probability of above-chance performance (p[Reward-Chance]) using generalized models. Task adaptation was successful; rats tracked shifting contingencies above chance (p[Reward-Chance]: Δ=0.04±0.005, p<.01). No sex differences emerged in p[explore] (p>.05), though females showed increased lose-shift behavior (Δ=0.04±0.017, p=.05) and longer RT (Δ=1.38±0.72, p=.05). PR stimulation reduced p[explore] vs. ON (Δ=0.35±0.006, p<.01) and OFF conditions (Δ=0.38±0.006, p<.01). DBS ON decreased lose-shift vs. OFF (Δ=0.15±0.036,p<.01) and PR (Δ=0.16±0.033, p<.01). Both ON (Δ=0.09±0.03, p=.05) and PR (Δ=0.27±0.03, p<.01) increased win-stay (reduced exploration after wins) vs. OFF, with PR reducing exploration vs. ON (Δ=0.18±0.03, p<.01). Finally, both ON (Δ=0.16±0.04, p<.01) and PR (Δ=0.21±0.04, p<.01) reduced RT vs. OFF. Our results reveal sex-specific explore-exploit approaches, with females demonstrating enhanced

lose-shift behavior. Mid-striatal DBS influences decision-making strategies condition-dependent, altering lose-shift/win-stay behaviors crucial for adaptive learning. Understanding these sex-specific and DBS-driven modulations offers a translational path towards refining neuromodulation therapies for cognitive inflexibility.

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Poster

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Topic: I.03. Decision Making

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University of Minnesota Doctoral Dissertation Fellowship

Title: Detangling the neural circuits of striatal deep brain stimulation

Authors: *E. M. SACHSE[1], E. M. DASTIN-VAN RIJN[2], M. BUCCINI[3], F. IACOBUCCI[3], A. S. WIDGE[3];
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Abstract: Neurostimulation of cortico-striato-thalmo-cortical (CSTC) circuits can ameliorate deficits in flexible decision-making. Deep brain stimulation (DBS) of the ventral internal capsule/ventral striatum (VCVS) in humans and DBS-like stimulation of mid-striatum (midSTR) in rats, both improve cognitive control, a component of healthy decision-making. Clinical DBS is thought to work by stimulating axons within the internal capsule, but the exact mechanisms remain unclear. By using optogenetic DBS and selective activation of axons vs. cell bodies we can determine which neural elements are the main mediators of DBS's behavioral effects. Rats (N = 36) were assigned to one of four different experimental groups. Groups 1 and 2 were transfected with AAV5-CaMKII-Chronos-GFP or AAV5-CaMKII-GFP in bilateral subregions of the mPFC. Groups 3 and 4 were transfected with AAV5-hSyn-Chronos-GFP or control virus in bilateral mid-striatum. All groups had optic fibers implanted in the mid-striatal electrical DBS target. Rats were trained on the Set-Shift task that requires cognitive control to successfully suppress more habitual responses. Rats then underwent a testing period of within-task bilateral stimulation ("opto-DBS", 470nm,~10mW, 130Hz, 2ms pulse width), and no stimulation (OFF condition) for a balanced number of ON/OFF sessions. Performance (response times (RT), accuracy, and omissions) was quantified using generalized linear mixed-effects models (GLMs)

to evaluate the effect of optical stimulation on cognitive control. Successful transfection and activation of Chronos was confirmed in a separate cohort of rats through neural recordings during opto-DBS and evoked-response potentials. Fiber localization and viral expression was confirmed by imaging. Opto-DBS of mPFC axons reduced RTs ($p = 0.001$) without increasing errors ($p = 0.1221$) in rats with active Chronos compared to control-virus rats. Opto-DBS of midSTR neurons increased RTs in Chronos rats compared to controls ($p = 0.0006$). Relative to their control groups, opto-DBS of mPFC axons decreased RTs more than opto-DBS of striatal neurons ($p = 1e\text{-}6$). There was some evidence that the reduction in RT from opto-DBS of mPFC axons diminishes over time, indicating that opto-DBS may not fully replicate effects of electrical midSTR DBS. Optogenetic DBS of mPFC axons improved cognitive control, replicating effects of electrical DBS. This indicates mid-striatal electrical DBS effects in rats are mediated by mPFC axons that project to and pass-through mid-striatum, as opposed to local striatal neurons. This supports the hypothesis that VCVS DBS in humans works through axonal stimulation.

Disclosures: E.M. Sachse: None. **E.M. Dastin-van Rijn:** None. **M. Buccini:** None. **F. Iacobucci:** None. **A.S. Widge:** None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR094.06/KK13

Topic: I.03. Decision Making

Support: ISF
ERC

Title: Mesoscale dynamics of action-outcome representations across the cortex in naturalistic goal-directed behavior

Authors: *T. CHAMILEVSKY[1], B. WANG[3], J. ALJADEFF[4], A. FINKELSTEIN[1,2];
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Abstract: Goal-directed behaviors involve action-selection and adjusting responses by observing action consequences. Here, we studied a novel naturalistic behavior consisting of multidirectional tongue-reaching movements towards a target to obtain a water reward (target presented on a grid of possible locations in front of the mouse's face). This behavior did not require training and allowed studying the underlying neural activity before it is shaped by learning. Using mesoscale 2-photon calcium imaging, we recorded the activity of ~1,000,000 neurons across >10 cortical areas including motor, somatosensory, and high-order visual regions. Cells were tuned to target location, reward outcome, and action time across all areas tested,

including conjunctive representation of all three variables. While target location selectivity was rather transient, the encoding of unexpected large reward persisted for >30 s across all cortical areas tested. Temporal dynamics within each cortical area showed rapid transitions between neuronal-ensembles encoding reward information. Specifically, reward modulation persisted on a population level, but was implemented dynamically by rapidly rotating dimensions in neural-activity space, that corresponded to specific neuronal ensembles with transient reward selectivity. The joint encoding of target location and reward modulation is consistent with a mathematical network model where these multivariate quantities are maximally mixed. We believe these long-lasting representations could indicate memory of action outcomes. We propose that the multiplexed nature of this memory could enhance its efficacy as a teaching signal for learning.

Disclosures: T. Chamilevsky: None. **B. Wang:** None. **J. Aljadeff:** None. **A. Finkelstein:** None.

Poster

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Topic: I.03. Decision Making

Support: R01NS120851
R01MH124687
R01MH123661
P50MH119569

Title: Sex-biased computations underlying differential set shift performance in mice

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Abstract: Cognitive flexibility is a key executive function, and can be defined as the ability to adaptively shift between choices or strategies based on environmental feedback. Maladaptive cognitive flexibility has been implicated in numerous neuropsychiatric conditions. Understanding drivers of altered cognitive flexibility between individuals can thus be poised to reveal mechanisms of neuropsychiatric risk and resilience. We demonstrate a newly-developed operant touchscreen Set Shift task that permits robust and continuous testing in mice, and use this task to interrogate sex differences in the computations supporting cognitive flexibility. This task requires animals to both commit to a rule-specific choice during periods of relative certainty (stable rule responding) and to flexibly explore alternative options during periods of uncertainty

(rule shifts). Using this task, we discovered that female mice completed significantly more rule shifts with fewer errors than males. We next employed a suite of computational models that revealed sex-biased individual differences in the computations underlying cognitive flexibility, including an input-output hidden Markov model (ioHMM) to label latent cognitive states. Animals occupy different cognitive states as they navigate the unsignaled changes in this environment-exploiting the current rule state and exploring to learn the new rule state following a rule shift. Cognitive flexibility can be formulated as the rate of adaptive transitions between exploration and exploitation. The most optimal strategy for the task is to rapidly transition to exploration following a loss on a previous rule, and transition to exploiting a new rule as rapidly as possible. We hypothesized that female mice were faster at at least one of these processes. Our results suggest that following rule shifts, female mice learn the new rule faster and commit to exploiting rule choices sooner compared to males-sometimes because they commit to multiple rules simultaneously.

Disclosures: N. Glewwe: None. **E.M. Dastin-van Rijn:** None. **C. Chen:** None. **E.M. Giglio:** None. **E. Knep:** None. **D. Mueller:** None. **B.A. Ebitz:** None. **A.S. Widge:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); unlicensed intellectual property related to deep brain stimulation. F. Consulting Fees (e.g., advisory boards); Abbott. **N.M. Grissom:** None.

Poster

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Topic: I.03. Decision Making

Support: P50 -MH119569
T32-MH115886
R01-MH123661

Title: Prefrontal cortex neuronal activity during explore and exploit states in a mouse bandit decision making task

Authors: *D. MUELLER[1], N. GLEWWE[2], E. KNEP[3], A. VELOSA[2], S. WEIR[2], U. MUGAN[2], A. YANG[1], B. A. EBITZ[5], N. M. GRISSOM[4];
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Abstract: The medial prefrontal cortex (mPFC) is known to regulate executive functions, such as decision making and cognitive flexibility. Across species, exploration represents periods of variable choice selection and heightened learning about the environment, while exploit behaviors show consistent, repeated choice selection that is less sensitive to trial-to-trial feedback. The mPFC has been identified as involved in mediating the tradeoff between exploration and

exploitation in complex tasks. We recorded from neural ensembles in mPFC via silicon probes in freely-behaving mice in a two arm restless bandit task, in which the reward probability of each choice changed randomly and independently across trials. Using a hidden Markov model (HMM), we identified explore/exploit states throughout restless bandit behavior. Previous research has demonstrated that rule states like exploration and exploitation are reflected in the global activity of the prefrontal cortex and at the level of single prefrontal neurons in primates. We asked whether mPFC ensembles differed between these states to encode different stages throughout the task. As the rules governing rewarded actions are established throughout a session of the bandit task, reward probabilities and cue information coded by mPFC neurons should increase, and we expect that correlated ensembles of neurons in this region will develop. To test these hypotheses, mice (129/b6j F1, 4 M, 4 F) were trained on two-arm spatial restless bandit schedules and implanted unilaterally with Cambridge Neurotech silicon probes (2 shank, 32 channels) directed to anterior cingulate and prelimbic cortex (AP +1.8, ML +/- 0.30, DV -2.05); single units were recorded and isolated using kilosort and phy. Units encoding outcomes, valence, and choices have been identified. We found that in bins of trials surrounding state transitions, there was more correlated activity in exploit-state labeled trials whether preceding or following an exploration state, suggesting greater neuronal synchrony in exploit states. Further, preliminary analysis with a time-resolved linear discriminant analysis indicates higher decoding accuracy versus chance in exploitation states compared to explore states. This research will provide insight into the specific decision states and mechanisms mediating complex decision making and individual decision making strategies.

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Poster

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Topic: I.03. Decision Making

Support: T32-NS105604 (MP)
5R01MH123661-05 (NMG)
1R01MH129370-01A1 (BTS)

Title: Dissociating the roles of nucleus accumbens and dorsomedial striatum dopamine in managing the explore-exploit tradeoff

Authors: *M. POROD[1], C. S. CHEN[2], A. PAULSON[2], B. A. EBITZ[3], B. T. SAUNDERS[1], N. M. GRISSOM[2];
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Abstract: Flexible decision making requires a fundamental tradeoff between exploiting options that are known to be rewarding and exploring amongst unfamiliar options to learn their value. Despite its central role in adaptive behavior, the neural mechanisms governing the explore-exploit tradeoff remain an open area of investigation. However, a growing body of evidence indicates that dopamine, through its well-established roles in reward learning, motivation, and action selection, plays a critical modulatory role. In the present study, we aimed to parse the contributions of dopamine signaling in the nucleus accumbens core (NAc) and dorsomedial striatum (DMS), two regions that have been ascribed distinct, but complementary behavioral functions. The NAc plays a key role in generating stimulus-outcome associations, whereas the DMS is linked to action-outcome learning. Therefore, we hypothesized that the NAc and DMS exhibit distinct dopamine signaling patterns during exploratory versus exploitative cognitive states. We trained male and female B6129SF1/J wildtype mice (n=24, 12/sex) on a touchscreen-based spatial two-arm restless bandit task in which the reward probability of each choice changes gradually and independently throughout the session. A hidden Markov model was used to identify latent exploratory versus exploitative choice sequences. Using fiber photometry with the dopamine biosensor dLight1.3b, we recorded dopamine activity in the NAc and DMS as mice engaged in the restless bandit task. Preliminary analyses indicate that dopamine activity in the NAc is modulated by state, exhibiting enhanced reward prediction error (RPE)-like dynamics during outcome presentation in explore states relative to exploit states. These results are consistent with the heightened learning demands associated with exploration. Ongoing analyses will characterize DMS dopamine signaling patterns. We expect that we will similarly observe enhanced RPE-like dopamine dynamics during explore relative to exploit states. Based on prior research, we expect to also observe enhanced movement-modulated signals during explore states, consistent with the heightened movement variability that has been observed during exploration. Together, these findings will clarify region-specific dopaminergic contributions to explore-exploit decision-making and provide insight into how striatal subregions differentially support flexible behavioral strategies.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Program #/Poster #: PSTR094.11/KK18

Topic: I.03. Decision Making

Support: DA050180
TL1TR001437
MHRC

Title: Molecular Mechanisms Underlying Discrete Components of Decision-Making: Brain Wide Disruption of system xC- Creates Deficits in Processing Punishment but Not Reward

Authors: *S. W. PECK[1], S. BOSE[2], G. J. SIMANDL[3], D. BAKER[2];
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Abstract: Impaired executive function underlies substance abuse disorders, obesity, and other chronic conditions, the treatment of which likely requires deeper insights into the underlying neurobiological mechanisms. Evolutionary enhancements in cognition have coincided with the emergence of novel molecular signaling pathways, which may indicate that specialized molecular signaling mechanisms are necessary for complex cognitive processes. Prior results indicate that system x_c^-, a cystine-glutamate antiporter expressed on astrocytes, is a recent evolutionary enhancement of glutamate signaling complexity that may contribute to phylogenetic gains in cognition. To further investigate this possibility, we assessed decision making in a touchscreen-based rodent gambling task by comparing wild type (WT) and genetically modified rats lacking Sxc function (MSxc). Optimal performance in the tasks requires rats to calculate reward and punishment magnitude and probability among four behavioral options. Over 15 sessions, WT rats learned to select the optimal stimulus 72% of the time while MSxc rats did not select the optimal stimulus above chance (27%), which resulted in a significant effect between genotype across sessions ($p < 0.05$). To deconstruct decision making, we assessed whether behavioral strategies optimized reward or minimized punishment, which are processes involving unique cortical regions. Across the 15 testing sessions, genotypic differences in behavioral strategies emerged such that WT were more likely to select options that minimized punishment relative to MSxc rats ($p = 0.026$). There were no genotypical differences in behavioral strategies designed to maximize rewards. Notably, impaired sensitivity to punishment with preserved reward processing was observed following a brain-wide elimination of Sxc activity, which has been shown to alter glutamate signaling in the cortex, striatum, hippocampus, and elsewhere, resembles the outcomes obtained with a localized disruption of the lateral orbitofrontal cortex, an area implicated in processing temporal punishments like those in the rodent gambling task. While further research is needed to integrate molecular and anatomical findings, this work suggests that specific molecular mechanisms may underlie specialized cognitive processes, akin to anatomical specialization in the brain. Identification and characterization of these mechanisms and their cognitive correlates could provide unique advantages, including accelerated development of new therapeutics and insights into biologically inspired computational models.

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Poster

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Program #/Poster #: PSTR094.12/KK19

Topic: I.03. Decision Making

Support: NIDA IRP Z1A-DA000633

Title: Exercise and Midbrain Dopamine Modulation of Impulsive Decision-Making in Mice

Authors: *H. SHEN[1], R. Y. KUANG[2], Y. CHAE[4], Y. DUAN[5], Z. MA[6], Z. XI[3], Y. YANG[7]; [2]Natl. Inst. on Drug Abuse, [3]Intramural Res. Program, [1]Natl. Inst. on Drug Abuse, Baltimore, MD; [4]NIDA IRP, Baltimore, MD; [5]Natl. Inst. on Drug Abuse, NIH, Nottingham, MD; [6]NIH, NIDA IRP, Baltimore, MD; [7]Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: Exercise is widely recognized for its benefits to physical and mental health, but its impact on cognitive performance and impulsive decision-making remains unclear. In this study, we examined how voluntary wheel-running influences impulsivity in adult male mice using the delay-discounting task (DDT), which measures preference for large delayed vs. small immediate rewards. Mice were classified into low- and high-impulsive groups based on baseline performance. Surprisingly, wheel-running increased impulsivity in low-impulsive mice - shifting their preference toward immediate, smaller rewards - while having no effect on high-impulsive mice. This suggests that exercise selectively increases impulsive choice in individuals with low baseline impulsivity. Given prior findings that wheel-running enhances midbrain dopaminergic neuron activity, we hypothesized a dopamine-dependent mechanism involved. To test this hypothesis, we used optogenetics to stimulate midbrain DA neurons non-contingently (25 Hz, 1-5 min) prior to the DDT. This mimicked the effect of wheel-running, increasing impulsive choices in low-impulsive mice. In contrast, contingent DA stimulation (25, 50 Hz, 20 sec) - delivered during each lever press for a large reward - decreased impulsivity in high-impulsive mice, without affecting low-impulsive animals. Thus, the behavioral effect of DA activation appears to depend on baseline impulsivity and whether stimulation is contingent or non-contingent. These findings reveal a nuanced relationship between exercise, dopamine signaling, and impulsivity: wheel-running and non-contingent DA activation promote impulsive decision-making in low-impulsive mice, while contingent DA stimulation reduces impulsivity in high-impulsive individuals. The mechanisms underlying these opposing effects remain to be determined.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

Location: SDCC Halls B-H

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Program #/Poster #: PSTR094.13/KK20

Topic: I.03. Decision Making

Support: KAKEN JP23K03006

Title: Anticipatory Negative Contrast in Instrumental Behavior: A cFos-Based Neural Analysis in Rats

Authors: *K. KAWASAKI;
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Abstract: Anticipatory Negative Contrast (ANC) is a phenomenon in which the value of a current reward decreases and associated behaviors (e.g., intake or response frequency) are reduced when an animal anticipates a better reward. For example, rats tend to avoid less sweet water if they know a sweeter solution will be available later. ANC is considered a model of behavioral inhibition, where individuals refrain from choosing a smaller immediate reward in favor of a larger delayed one. Mechanisms such as anticipation, reward comparison, and behavioral inhibition underlie this behavior. Since this form of self-control is often impaired in young children and individuals with Attention Deficit Hyperactivity Disorder (ADHD), elucidating the underlying neural mechanisms is vital to understanding human anticipatory behavior. Previous research has primarily studied ANC in consummatory behaviors, such as licking sucrose solutions, while instrumental behaviors like lever pressing have not been examined. Since many human behaviors are instrumental, confirming ANC in instrumental behavior is necessary to investigate brain mechanisms as a human model. In our previous study of nose-poking behavior, we were the first to show that ANC can also be observed in instrumental behavior. Thus, the current study aims to identify brain regions involved in ANC mechanisms in instrumental behavior via cFos expression analysis. In the first experiment, rats were trained to perform a nose-poking task. During the first component, all rats followed a fixed ratio 3 (FR3) schedule and received a single 20 mg pellet as a reward. In the second component, the control group continued with the FR3 schedule and received one pellet, whereas the experimental group followed an FR1 schedule and received three pellets. The average number of nose-poking actions significantly decreased in the experimental group during the first component, indicating the presence of ANC. Ninety minutes after the sixth or tenth training day, brain tissue was collected to examine cFos expression in the prefrontal cortex, amygdala, and insular cortex. However, no significant differences were observed between the groups in any of these regions. In the second experiment, a similar training procedure was used. Brains were collected 90 minutes after training on either the second or fourth day. In contrast to the first experiment, cFos expression was significantly elevated in the experimental group across all examined regions, irrespective of training duration.

Disclosures: K. Kawasaki: None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Topic: I.03. Decision Making

Support: NSF IOS 2137023
NIH/NIMH R01MH132018-02

Title: Ventral Tegmental Area glutamatergic projections to the hippocampus guide encoding of contextual memory

Authors: *S. SINZA[1], O. M. OGUNDELE[2];
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Abstract: Reciprocal neural circuit between the ventral tegmental area (VTA) and the hippocampus governs aspects of learning and processing of information for long-term cortical storage. VTA controls reward, aversion, addiction, etc. Similarly, VTA contains diverse neuron types, including dopaminergic, glutamatergic, and GABAergic majorly. VTA glutamatergic terminals in the hippocampus are the dominant type (66-85%). The role of VTA glutamatergic projections in the hippocampus contribute to the learning of reward is less studied. Here, we investigated VTA glutamate terminal innervation of the hippocampal CA1 region and its role in encoding spatial learning and contextual memory. To test the functional implication of VTA glutamate innervation to CA1, light-controlled excitatory opsin was expressed in VTA glutamate neuron projections by stereotaxic injection of AAV-DIO-ChR2 into the VTA of *Vglut2*^{cre} mice (n=5). To assess the VTA-CA1 glutamate tract in mice, a fiber-optic cannula was positioned in the CA1, while neural probe shanks were positioned in the dorsal CA1 for recording. A baseline Conditioned Place Preference (CPP) test was performed without neural modulation for 4 days (Preconditioning, Conditioning for 2 days, and Test Day) with 16% sucrose reward. 10% vinegar was used as a vehicle. Two different types of tactile cues were used in the CPP chamber for the two different sides. Before this test, habituation and food restriction were carried out. After 1 week, the same CPP task was performed with optogenetic activation of the VTA glutamate projections for 4 days with switched cues. Light was activated when the animal chose the reward side. Behavioral tasks, CA1 recording, and photo-modulation were synchronized by behavioral tracking and hardware control TTL systems. Plexon OFSS and Neuroexplorer were used to analyze electrophysiological data. Statistical analyses were done for behavioral data analysis by Origin Pro 2024, where a normality test was done, and ANOVA repeated measure, followed by Dunnett post hoc test, along with a paired t-test, were performed. The outcome of this experiment will determine the role of VTA glutamate inputs in CA1 in the integration of spatial memory and context discrimination.

Disclosures: S. Sinza: None. **O.M. Ogundele:** None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Program #/Poster #: PSTR094.15/KK22

Topic: I.03. Decision Making

Support: DA043443

Title: Circuit-specific dopaminergic modulation of probabilistic reversal learning via chemogenetic activation of VTA projections

Authors: B. CHO[1], **G. S. THIBODEAU**[1], E. BURKE[2], H. SANCHEZ[3], *J. R. TAYLOR[4]; [1]Psychiatry, [3]Div. Molec Psychiatry, [2]Yale Univ., New Haven, CT; [4]Yale Univ. Sch. Med., New Haven, CT

Abstract: Previous studies using the probabilistic reversal learning (PRL) task have demonstrated that rats with poor reversal performance exhibit lower positive-outcome updating and increased midbrain D3 receptor binding potential (BPND), relative to good reversal performers. In parallel, ablation of the amygdala to orbitofrontal cortex (OFC) neurons impairs reversal performance and disrupts value updating after a positive outcome. Building on this framework, we hypothesized that chemogenetic activation of ventral tegmental area (VTA) projections to either amygdala or OFC would enhance the behavioral response to reversal and promote more adaptive reinforcement learning. To test this, we infused animals with a Cre-dependent excitatory DREADD virus targeting VTA dopamine neurons, along with a retrograde Cre recombinase virus in either the amygdala or the OFC. Once behavioral performance stabilized on the PRL task, rats underwent [^{11}C]-(+)-PHNO PET imaging under CNO (1mg/kg) or vehicle to assess dopaminergic receptor occupancy (BPND). Following PET imaging, rats underwent the PRL task for 7 days while the targeted neural circuit was activated. In order to do this, rats received CNO/vehicle for 7 consecutive days, administered 30-min before the PRL task, thereby selectively activating the targeted circuit during the PRL task. Activation of the VTA-to-amygdala projection did not improve reversal performance in either good or poor reversal rats (i.e., number of achieved reversals/session). However, assessments of performance using trial-by-trial choice data fit to a reinforcement-learning model (i.e., positive and negative outcome updating) revealed that CNO administration significantly increased positive-outcome updating and also the probability of staying with a reward response in poor reversal rats. No differences were observed in negative-outcome updating or the probability of switching after unrewarded trials. These findings suggest that augmenting dopaminergic activity in VTA-amygdala circuits may selectively boost reinforcement sensitivity in animals with suboptimal learning strategies, but activation of only the VTA-to-amygdala circuit is not sufficient to facilitate behavioral shifts toward more adaptive decision-making. Ongoing analyses of the VTA-to-OFC manipulation will determine whether additional circuit mechanisms are required for improved reversal learning performance.

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Poster

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Topic: I.03. Decision Making

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Title: Contributions of monkey substantia nigra pars reticulata during perceptual decision making

Authors: *J. BHATTI[1], K. BRANAM[2], J. I. GOLD[2], L. DING[2];
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Abstract: How the basal ganglia (BG) contribute to noisy evidence-based perceptual decision making is not yet well understood. For example, little is known about the computational roles of the major output nuclei of the BG, the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). A previous study of GPi identified neural representations of already-formed choices but not, for the most part, evidence-related quantities. We hypothesize that the SNr contributes to decision formation based on noisy evidence.
To test this hypothesis, we recorded single-unit SNr activity in two monkeys performing a visual motion-discrimination task. The monkeys made saccadic eye movements at a self-chosen time to indicate their decision about the direction of a random-dot motion stimulus. For each trial, the motion strength and direction were chosen randomly from five values and two directions, respectively. We identified 107 SNr neurons with heterogeneous activity patterns after motion stimulus onset and/or around saccade onset. Using multiple linear-regression analyses, we found that ~45% of neurons showed choice-selective activity in the 300ms window leading up to saccade onset, of which approximately half (25 of 48 units) showed greater activity for trials with contralateral choices. A large fraction of SNr neurons (~57%) showed modulation by motion strength and/or reaction time. Clustering analysis suggests that our SNr sample contains at least three distinct populations, each with different combinations of choice and motion strength modulation.
We applied electrical microstimulation to the SNr to test for a causal link between its activity patterns and decision-making behavior. Out of 52 sessions, SNr microstimulation significantly biased the monkeys' saccadic choices in 23 sessions, with contralateral and ipsilateral biases observed in 17 and 6 sessions, respectively. We also observed significant changes in response time (RT) for ipsilateral choices in 20 sessions (18 of which showed increased RT) and for contralateral choices in 16 sessions (8 of which showed increased RT). In 36 sessions, the microstimulation effects were reflected in changes in at least one parameter in a drift-diffusion model (DDM) with collapsing bounds. These results support the idea that the SNr does not simply represent already-made choices but instead contributes to a decision process involving evidence accumulation and decision commitment.

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Poster

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Topic: I.03. Decision Making

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Title: Dynamic Reorganization of High-Dimensional Cortical Activity During Goal-Directed Behavior

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Abstract: Cognitive processes unfold over a wide range of spatial and temporal scales, engaging activity across multiple brain areas. Recent studies have begun to characterize spatiotemporal dynamics across the neocortex (Stringer et al., 2019; Manley et al., 2024), yet little is known about how cortex-wide activity at the level of individual neurons is organized to support behavior. To address this gap, we used mesoscale calcium imaging (Sofroniew et al., 2016) to record activity from up to ~30,000 neurons simultaneously (~1,000,000 neurons total) across more than 10 cortical areas, including somatosensory, motor, and higher-order visual regions. Recordings were obtained while mice performed a goal-directed behavior requiring multidirectional tongue-reaching for water rewards. Both global (inter-areal) and local (intra-areal) cortical dynamics were high-dimensional during the task, with variance distributed broadly across dimensions. Notably, global cortical dynamics during spontaneous behavior had even higher dimensionality - a difference that could reflect either local dimensionality quenching or enhanced inter-areal coupling during task performance. We found that local dimensionality remained similar across states, despite largely distinct activity fluctuations, with only a small subset of dimensions shared between spontaneous and task-driven dynamics. During task engagement, a transient quenching of dimensionality occurred reliably around reward time in all cortical regions, suggesting temporal coordination. In addition, we identified high-dimensional communication subspaces linking cortical areas (Semedo et al., 2019) in both states, with significantly increased inter-areal communication during task performance. Finally, task-related movements engaged a high-dimensional activity space with broad anatomical distribution, which interacted with inter-areal communication subspaces in a distance-dependent manner, suggesting spatial gating of movement-related information. Together, our results reveal that high-dimensional, distributed cortical activity and dynamic inter-areal communication reorganize in a structured manner to support goal-directed behavior.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR094.18/LL3

Topic: I.03. Decision Making

Support: ISF
ERC
CRCNS NSF-BSF

Title: Functional organization of motor cortex subnetworks underlying naturalistic reaching behaviors

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Abstract: Goal-directed behaviors involve coordinated activity of neurons encoding various cognitive variables of decision-making, such as target-selection, movement timing, and reward outcome. In mice, the anterior lateral motor (ALM) cortex plays a central role in goal-directed licking behaviors. However, it remains unclear how these distinct cognitive components are integrated at the level of neural function and the underlying connectivity. To address this, we used a novel naturalistic tongue-reaching behavior that requires no pre-training, enabling us to study network-level interactions among neurons from the very first day of learning. Using two-photon calcium imaging of 100,000 excitatory neurons in layers 2/3 of ALM and unsupervised clustering we found stereotypical neural responses encoding target-location, movement timing and reward outcomes. We hypothesized that neurons belonging to the same cluster (as defined during the task) would exhibit stronger functional coupling during spontaneous activity, potentially reflecting shared connectivity. Consistent with this hypothesis, we found that removing the leading components of population activity ('shared subspace') from the spontaneous neural activity using dimensionality reduction, revealed robust functional coupling within clusters and diverse cooperation or competition across clusters, suggesting recurrent interactions that were hitherto masked by a shared subspace. The strongest functional coupling was found between neurons that responded later in the post-movement window - potentially suggesting larger influence of recurrency during reward-outcome processing. This functional motif emerged from the first day of behavior, and became more pronounced with experience. To causally probe recurrent interactions, we employed optogenetic stimulation of single neurons, which revealed neurons with sustained response following photostimulation. Interestingly, these neurons were most active near movement onset. Together, our findings demonstrate how motor cortex subnetworks encode action-relevant cognitive variables and adapt with experience, laying the groundwork for uncovering the recurrent circuit mechanisms underlying naturalistic, goal-directed behavior.

Disclosures: N. Shmueli: None. **A. Finkelstein:** None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR094.19/LL4

Topic: I.03. Decision Making

Title: Dopamine signals transmitted to different subregions of the striatum in macaque monkeys performing a value-based decision-making task

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Abstract: Midbrain dopamine neurons are widely recognized for their role in decision-making, particularly through encoding reward prediction errors. According to the brain's reinforcement learning framework, these dopamine signals are transmitted to the striatum, where they modulate cortico-striatal synaptic plasticity. This modulation is thought to reinforce actions leading to better-than-expected outcomes and suppress those leading to worse-than-expected ones. While this model has received broad empirical support, recent findings challenge the notion that dopamine neurons form a uniform population dedicated solely to encoding reward prediction errors. Instead, they appear to consist of functionally distinct subpopulations involved in signaling salience, regulating motor behavior, and supporting cognitive processes. These observations suggest that the role of dopamine in decision-making is more diverse and complex than previously assumed. To understand circuit level mechanisms underlying decision-making, it is essential to identify the precise signals transmitted from dopamine neurons to the striatum and to elucidate their roles in computations for decision-making. To address this, we used a genetically encoded dopamine sensor, dLight, to measure dopamine release in the striatum. While widely applied in rodents, such sensors have been less frequently used in non-human primates. In this study, we employed dLight in macaque monkeys, a well-established model for human executive function, to track dopamine signals transmitted to the striatum during a value-based decision-making task. This task allowed us to continuously monitor dopamine signals throughout option evaluation, choice selection, and reassessment of past decisions. We found that distinct striatal subregions, the caudate nucleus and putamen, receive different dopamine signals that may serve unique roles in decision-making. Dopamine signals in the putamen encoded reward prediction errors associated with decision outcomes, while those in the caudate nucleus reflected option value, choice selection, and counterfactual (unchosen) option information—critical for reassessing prior decisions. By demonstrating parallel pathways

transmitting distinct signals, our study highlights the broader role of dopamine neurons-striatum circuitry in decision-making.

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Poster

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Program #/Poster #: PSTR094.20/LL5

Topic: I.03. Decision Making

Support: ELSC Grant
Azrieli ICAMH fellowship

Title: Uniform dopaminergic coding of reward prediction and subjective value along the caudate in monkeys

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Abstract: We used fiber photometry to record sub-second dopamine (DA) signals from the striatum of monkeys performing tasks involving varying reward sizes and probabilities, to examine how DA encodes key aspects of decision-making. DA signals conformed to reward prediction error (RPE) theory: at cue presentation, DA levels increased in proportion to the predicted reward's size and probability. At reward delivery, DA responses were inversely related to outcome predictability, with stronger responses for surprising outcomes. This RPE coding mostly reflected subjective value: both behavioral preferences and DA signals favored risky options offering a large potential reward over safer ones with equivalent expected value, indicating internal valuation beyond objective utility. Furthermore, DA signals encoded RPEs in an abstract and uniform manner. We found overall homogeneity in responses along 10 mm of the anterior-posterior axis of the caudate. Responses related to the laterality of the visual cue or the direction of movement were very small, and there was no correlation between movement timing and DA signals, underscoring its specificity for reward-related processing rather than directly controlling behavior. During choices between alternative options, DA signal remained consistent across different pairings when the same high-value option was presented against lower-value alternatives. In error trials, where the monkey selected the lower-value option, the cue response still resembled that associated with the higher-value, unchosen offer, indicating that early DA activity encodes offered value. Only after incorrect choices did DA levels show a marked decrease. This response occurred even in the absence of explicit feedback, reflecting an internal signal that negates the animal's own choice, pointing to a dopaminergic mechanism for self-evaluation or decision-related conflict.These findings provide critical insights into dopamine's

role in encoding expected and subjective value, tracking prediction errors, and supporting internal performance monitoring in primate decision-making.

Disclosures: Y. Henshke: None. **E. Lottem:** None. **M. Joshua:** None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Program #/Poster #: PSTR094.21/LL6

Topic: I.03. Decision Making

Support: MnDRIVE Graduate Fellowship

Title: Multi-parameter optimization of VCVS Deep Brain Stimulation using objective cognitive control

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Abstract: Deep brain stimulation (DBS) of the ventral internal capsule/striatum (VCVS) represents a potential therapeutic option for various mental health disorders in cases where conventional treatments have proven ineffective. However, the efficacy of DBS relies on accurate programming that targets the specific sub-circuits within VCVS. Presently, VCVS programming is a time-consuming and iterative process, requiring weeks between adjustments and relying on subjective self-reports that can be prone to noise. Introducing an objective measure of circuit engagement could enable rapid testing of individual settings within seconds to minutes, significantly reducing the time required to achieve a response and instilling greater confidence in the selected settings for both patients and clinicians.
In our previous investigation, we developed a simulation framework that utilized an objective measure of reaction time derived from the Multi-Source Interference Task—a cognitive control task. This framework, integrated with an adaptive optimization algorithm, enabled a systematic exploration of electrode contacts to identify those that yield the greatest improvements in cognitive control. We also presented empirical evidence demonstrating the superior performance of the Upper Confidence Bound (UCB1) algorithm over other optimization methods. Using a majority-vote ensemble approach, this implementation achieved approximately an 80% probability of converging to the global optimum, with the stimulation site as the primary parameter of interest. The current study extends this framework by incorporating additional stimulation parameters—namely, amplitude and pulse width—into the optimization process. These parameters play a critical role in modulating the efficacy and specificity of deep brain stimulation (DBS). By optimizing not only the stimulation site but also the intensity and temporal characteristics of the stimulation, this extension aims to refine the parameter selection process. The inclusion of amplitude and pulse width enhances the granularity of the optimization

space, thereby increasing the likelihood of identifying parameter combinations that maximize cognitive performance. This broader approach holds promise for improving both the precision and reliability of DBS programming.

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Poster

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NIH D-SPAN Fellowship 5K00NS105204-05

Title: Intracranial traveling waves during sequential inspections in a multi-attribute decision task

Authors: *C. C. LI[1], A. L. SAMPSON[2], E. E. EMERIC[3], W. J. LIPSKI[6], S. MOREIRA GONZALEZ[8], A. DAMIANI[7], V. STUPHORN[2], S. V. SARMA[4], J. A. GONZÁLEZ-MARTÍNEZ[6], E. NIEBUR[5];
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Abstract: Traveling waves, both spontaneous and stimulus-evoked, have been found to propagate in human and simian cortex (Davis et al., 2020, *Nature*; Muller et al., 2014, *Nature Communications*). Past work has focused primarily on waves that travel along the cortical surface. Using intracranial stereo-electroencephalography (sEEG) with electrodes that penetrate into deeper tissues, we aimed to also detect waves traveling along the medial-lateral axis of the human brain. We recorded the neural activity of six epilepsy patients while they engaged in a multi-attribute decision-making game on a tablet. In each trial, patients were presented with two options, each associated with a monetary amount and a probability of either winning or losing it. Participants examined the two attributes of each option by unmasking them one at a time before making a selection. This inspection period required sequential information accumulation and encoding of recent history, a process for which traveling waves are hypothesized to be a mechanism (Keller et al., ICLR 2024). In accordance with past research, we filtered the local field potential recorded at each contact into the 5-40 Hz range, computed the phase angle of the signal (Davis et al., 2020), and isolated 0.5-second windows aligned to inspection events (i.e., attribute unmasking times) of the task. Within these epochs we surveyed for structured wave propagation of multiple types. By focusing on recording contacts near the cortical surface on multiple sEEG electrodes we can look for waves that traverse the cortical surface. Within

individual electrodes—each of which consists of 8-18 evenly spaced recording contacts—we can identify subsets of spatially adjacent contacts in which the timing of wave peaks varies linearly with a contact's position on the electrode. In medially to laterally propagating waves the temporal latency between a reference time point and the time of peak phase increases with the contacts' distance from the center of the brain, and in medially to laterally propagating waves we would observe the opposite. To measure such waves, we test for a significant circular-linear correlation (Mardia, 1976, *Biometrika*) between the phase of a signal recorded at a contact and the contact's distance from the end of the electrode. Preliminary results show medial-lateral traveling waves in brain areas including the orbitofrontal cortex, a region associated with assessing reward value.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR094.23/LL8

Topic: I.03. Decision Making

Support: NSF Grant BCS2237795

Title: Spatiotemporal Decoding of Reward in Human Cortex Via Magnetoencephalography

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Abstract: Humans use reward-based learning to guide adaptive decision-making. While fMRI has identified brain regions sensitive to reward, its limited temporal resolution hinders characterization of the spatiotemporal dynamics underlying reward-guided decisions. In this study, we make use of the combined spatial and temporal resolution of magnetoencephalography (MEG) to uncover the dynamic evolution of neural computations involved in reward-guided decision-making. Specifically, N=26 participants completed a probabilistic reinforcement learning task (i.e., a 3-armed Bandit task) during MEG scanning with the goal of maximizing rewards. Participants also completed structural MRI scans to enable source-space decoding of reward-locked MEG responses via minimum norm estimation (MNE). The task comprised 224 trials with ≈50% reward probability, providing substantial within-subject power for decoding reward versus non-reward responses. We then deployed dynamic statistical parametric mapping and machine learning to estimate spatiotemporally-resolved neural signatures of reward. This analysis yielded robust reward decoding (AUC) in early visual areas (≈150-200ms post-feedback) and a distributed network of prefrontal and limbic cortical regions (≈450-500ms post-

feedback). Building from these results, we are present conducting follow-up analyses to examine how subject-specific reward decoding in each region identified at the group-level can predict subsequent decisions to maximize rewards (i.e., exploitation) versus sampling distinct options in the task environment (i.e., exploration). The ultimate aim of this work is to provide a clearer picture of the emergence of reward responses in the human brain, with direct relevance for understanding brain disorders associated with pathological reward-guided decision-making.

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Poster

PSTR094: Neural Mechanisms: Choice in Reward

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Topic: I.03. Decision Making

Support: NRF-2022R1A2C1010704

Title: Cerebellar and Cortical Functional Connectivity in Delay Discounting

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Abstract: Heightened impatience for temporal delays is a critical factor in decision-making, contributing to several psychiatric disorders characterized by impulsivity. Delay discounting (DD), the devaluation of future rewards with increasing delay, is a key behavioral measure of this impatience. Prior research has identified brain regions within the valuation [medial prefrontal cortex, ventral striatum (VS), posterior cingulate cortex], salience [anterior cingulate cortex (ACC), anterior insula (aINS)] networks, and prospective (medial temporal regions) networks as correlates of DD. Emerging evidence also implicates the cerebellum in a range of cognitive functions beyond motor control. However, the specific role of the cerebellum in DD remains largely unexplored. Therefore, this study investigated the relationship between functional connectivity (FC) within the cerebellum and between cerebellar subregions and components of the valuation and salience networks in relation to individual differences in DD. Resting-state fMRI data were acquired from 57 participants. Individual DD rates were assessed behaviorally. We examined FC using cerebellar subregions and regions within the valuation and salience networks to identify associations with individual DD rates. Results revealed a significant negative correlation between the FC of the cerebellar Crus II region and the ACC with DD. Conversely, positive correlations were observed between DD and the FC of the aINS with the ventral striatum, as well as between the ACC and the amygdala. These findings suggest a novel role for the cerebellum, particularly the Crus II region and its connectivity with the ACC, in individual DD. Furthermore, our results corroborate the involvement of functional coupling

within the established valuation and salience networks in individual differences in DD. Specifically, stronger connectivity between regions involved in salience detection (aINS) and valuation (VS), as well as between regions involved in salience and emotional processing (ACC and amygdala), was associated with higher rates of delay discounting (greater impulsivity). In contrast, increased connectivity between a cerebellar region implicated in cognitive control (Crus II) and a key salience network node (ACC) was associated with lower impulsivity. These findings provide new insights into the neural mechanisms underlying impulsive choice behavior in individuals exhibiting high DD rates and may inform future research on potential neuromodulatory targets for interventions aimed at reducing impulsivity.

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Poster

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Program #/Poster #: PSTR094.25/LL10

Topic: I.03. Decision Making

Support: ZIA MH002893

Title: Temporal decoding of reward processing using MEG

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Abstract: The Reward Positivity (RewP) is a sensitive marker of reward, typically measured at frontocentral electrodes. The RewP is likely generated by a distributed network of brain regions that do not contribute homogenous information. Given the broad set of contributors to this signal, using a univariate approach such as ERP analysis may not capture the full scope of this signal. Multivariate pattern analysis (MVPA) has shown increased sensitivity to neural signals and allowed for successful classification of perceptual stimuli based on evoked neural activity. MEG data suggest that we can use MVPA to temporally decode rewarding and non-rewarding feedback in individual participants. Participants completed a reinforcement learning task in which they received a blue or yellow screen that indicated monetary reward. Feedback-color mapping changed between runs. MVPA was first used to classify color at each timepoint, irrespective of feedback. This sanity check model accurately predicted color from 70-250ms. Feedback was accurately predicted from 200-800ms in a model including both blue- and yellow-reward trials. A cross-decoding model, in which the training set included rewards of one color and the testing set included rewards of the other color, made logical systematic errors early in the trial (70-250ms) in line with color processing, but successfully predicted feedback later in the trial (350-800ms). This suggests that we are successfully decoding the response to the cognitive content of the feedback, not just the perceptual attributes. This method could allow for differential decoding of both perceptual and cognitive attributes of reward. Additionally, the

sensitivity of the method could allow for better understanding of individual differences in reward processing.

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Poster

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Topic: I.03. Decision Making

Support: NIH U01NS132788
NIH U01NS117839

Title: Microstimulation of human substantia nigra compacta modifies loss aversion and increases momentary happiness

Authors: *M. YEBRA[1], C. P. MOSHER[2], S. CHENG[1], H. COURELLIS[4], Z. FU[5], A. MAMELAK[1], U. RUTISHAUSER[3];
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Abstract: Computational modelling has demonstrated that the experience of happiness in a probabilistic reward task is determined by a combination of expected reward and reward prediction errors (RPE), with RPEs explaining most variance (Rutledge et al., 2014). To test this model causally, we hypothesized that microstimulation of dopaminergic neurons in the Substantia Nigra (SN) would manipulate RPEs, and therefore subjective happiness. We conducted behavioral experiments while stimulating and recording from 101 single neurons in SN in 24 Parkinson's patients undergoing deep brain stimulation (DBS) surgery. Subjects performed a probabilistic reward task, choosing between a certain option and a gamble with two equiprobable outcomes, and rated their happiness every three trials. Stimulation (130 Hz, 0.03 mA) was delivered in blocks of three trials. Patients' behavior reflected strategic value-guided decisions: they were more likely to gamble when the gamble's expected value (EV) exceeded the certain reward (CR). This effect was selective to gain trials and absent for losses, consistent with goal-directed rather than indiscriminate risk-taking. Fitting a Prospect Theory model to the behavior revealed that SN stimulation caused a significant reduction in loss aversion (λ), with no difference in risk aversion (α), suggesting specific recalibration of value asymmetries. This led to subjects increasing their selection of high-variance gambles only when they were objectively favorable (EV > CR) when stimulted, leading to higher overall earnings. Fitting a happiness model to the happiness ratings given confirmed that RPEs best predicted moment-to-moment happiness, and that adding stimulation improved model fit, indicating affective amplification via enhanced RPEs. Neuronal analyses revealed that 17/101 neurons encoded the maximal possible loss during the offer screen. The activity of these neurons was

modulated by prior stimulation, pointing to a mechanism for how SN stimulation leads to reduced loss aversion. While 29/101 neurons encoded RPEs and 28/101 tracked happiness, these populations did not overlap, indicating that the substrate of the two is separate. Happiness neurons were modulated by RPE across trials, suggesting indirect affective updating via RPE signals. Altogether, this causal test of the effect of dopaminergic neuronal activity in the human SN revealed that stimulation recalibrates loss sensitivity, promoting greater engagement with high-EV gambles that yield enhanced RPEs, which in turn elevate happiness. Thus, stimulation modulates the computations that shape emotion via action and outcome, rather than directly shifting mood.

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Poster

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Topic: I.03. Decision Making

Support: JSPS KAKENHI Grant Number JP23K11788

Title: The self-determination of consideration rules in the ultimatum game enhances activity in the retrosplenial cortex

Authors: *N. MIURA[1], Y. HAMAMOTO[2,3], R. EMURA[4], S. OGATA[5], M. SUGIURA[6,7]; [1]Tohoku Inst. of Technol., Sendai, Japan; [2]The Frontier Res. Inst. for Interdisciplinary Sci., [3]Res. Inst. of Electrical Communication, [4]Grad. Sch. of Arts and Letters, [5]Cognitive Neurosci. Application Ctr., [6]IDAC, [7]Intl. Res. Inst. of Disaster Sci., Tohoku Univ., Sendai, Japan

Abstract: We find it difficult to tolerate unfairness imposed by others, but we tend to accept unfairness in which we were involved in making the decision ourselves. For example, if you make a rule to divide snacks and let your family choose first, you might get the smallest share, but you may accept it because you set the rule yourself. Thus, it is expected that people's judgments of gains and losses are not based solely on simple profit and loss, but also consider the constructed situation. In particular, the extent to which one is voluntarily involved in constructing the situation may influence their judgment. In this study, we used fMRI to examine how having the opportunity to self-determine unequal rules between groups during the ultimatum game with multiple group members influences brain activity when deciding whether to accept a monetary offer. Seventy-nine healthy Japanese university students participated as responders in an ultimatum game. Proposers were virtual participants assigned to either a young or elderly age group. In addition to standard ultimatum game rules, allocation amounts were adjusted to benefit a specific age group, for example, under a youth-priority rule, young proposers received more advantageous offers. Participants were divided into two groups: a self-determination group,

where they chose which proposer age group received favorable allocation rules, and an experimenter-assigned group, where these rules were predetermined. We used SPM and the Sandwich Estimator toolbox for analysis. Using parameter estimates when accepting offers from proposers in advantageous or disadvantageous positions according to the allocation rule, we compared brain activity between the self-determination and experimenter-assigned groups. Significantly greater brain activity was found in the retrosplenial cortex and right posterior cerebellum in the self-determination group compared to the experimenter-assigned group. Additionally, a negative correlation was found between neuroticism and activity in the retrosplenial cortex when accepting advantageous offers from the proposer. The two groups differ only in whether they have self-determination with respect to the considerate rule; accordingly, it is concluded that self-determination affected their attitude toward engaging with the task. Moreover, in relation to neuroticism, it can be suggested that the change in attitude triggered cognitive mechanisms that regulate emotional responses to unfair allocations, reflected as group differences in neural activity.

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Poster

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Support: URCA Grant FR-2024-1034

Title: The neural basis of the human justice intuition.

Authors: *A. MOLINARO[1], M. OKHAN[2], A. Q. MA DE SOUSA[1], D. SZNYCER[3], H. YU[1]; [1]Dept. of Psychological and Brain Sci., Univ. of California Santa Barbara, Santa Barbara, CA; [2]Univ. of California Davis, Davis, CA; [3]Dept. of Psychology, Oklahoma State Univ., Stillwater, OK

Abstract: The success of human societies relies on large-scale cooperation among non-kin, a feat attributed to the establishment and enforcement of social norms. Often, legal codes uphold these norms through punishing behaviors that deviate from collective societal interests. For this socially reliant system to function properly, evolutionary psychologists posit that over time selection pressures built upon domain-general neural networks to develop a shared set of cognitive tools for evaluating the morality of behaviors in a consistent manner. We propose that these cognitive adaptations form the basis of the “human justice intuition” - humans’ shared, innate sense of what is right and wrong. This is supported by numerous studies demonstrating that across cultures, people robustly agree about the deserved punishment of various crimes. This study investigated the neural basis of this intuition by replicating a behavioral study involving

third-party punishment for various crimes (Sznycer & Patrick, 2020) while collecting fMRI data. Twenty-one U.S. college students rated 55 criminal offenses on four dimensions while in scanner: (1) Shame - how much shame they would feel if they committed the offenses, (2) Devaluation - how much they would look down upon someone who commits them, (3) Wrongness - how morally wrong the offenses are, and (4) Time - how much prison time someone deserved for committing them. Using Representational Similarity Analysis (RSA), we examined how neural activation patterns in the dorsal anterior insula (dAI) and the posterior temporoparietal junction (TPJ) corresponded with participants' ratings across all conditions. Results showed that neural activation patterns in both the dAI and TPJ significantly tracked participants' prison time ratings. Furthermore, dAI activation during devaluation and shame judgments, and TPJ activation during shame judgments correlated with participants' prison time ratings. These findings suggest that the neural representations of shame and devaluation play a critical role in the cognitive processes underlying third-party punishment. Supporting adaptationist theories, this study provides initial neural evidence for a shared cognitive architecture underlying moral evaluation and norm enforcement through third-party punishment.

Disclosures: **A. Molinaro:** 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.; I received an Undergraduate Research & Creative Activities (URCA) grant for this project.. **M. Okhan:** None. **A.Q. Ma de Sousa:** None. **D. Sznycer:** None. **H. Yu:** None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR094.29/LL14

Topic: I.03. Decision Making

Support: NIMH R01 MH076136 (Tor Wager)

Title: Pain, tDCS, and Decision-Making: The Influence of Social Context and Task Repetition

Authors: A. DEHGHANI, ***D. GANTZ**, T. D. WAGER;
Dartmouth Col., Hanover, NH

Abstract: This study examines altruistic or egoistic behavior in a decision-making task when people are offered a choice to either reduce one's own pain or the pain of another person, versus opting for personal gain. We addressed this question across two studies. In the first study, participants engaged in a social decision-making task where they chose to accept or reject a monetary payoff that caused pain to themselves or another person, which was repeated two times to assess choice consistency. In the second study, participants received anodal, cathodal, and sham transcranial direct current stimulation (tDCS) over the left motor cortex (M1) before completing the same decision-making task, allowing for an investigation of how tDCS influences

decision-making and pain valuation, as well as the consistency of altruistic and egoistic behavior by participants. Results of the first study showed significant effects of pain ($T(198) = -13.8$, $p < 0.001$, higher pain offers increased probability of refusal), monetary value ($T(198) = 15.7$, $p < 0.001$, higher monetary offers increased probability of acceptance), and hyperaltruistic behavior by participants (Decision type $T(198) = 3.7$, $p < 0.001$, accepting more offers for self vs. others). However, the level of altruistic behavior decreased after repeating the decision-making task. Results of the second study showed significant effects of pain ($T(150) = -11.8$, $p < 0.001$, higher pain offers increased probability of refusal), monetary value ($T(150) = 12.1$, $p < 0.001$, higher monetary offers increased probability of acceptance), hyperaltruistic behavior by participants (Decision type $T(150) = 3$, $p < 0.01$, accepting more offers for self vs. others), a significant interaction between cathodal and pain ($T(150) = 2.6$, $p < 0.05$, under cathodal stimulation, the negative effect of pain on acceptance became less negative), and a marginal interaction between Decision type and cathodal stimulation ($T(150) = -1.9$, $p = 0.059$, under cathodal tDCS, participants are less likely to accept offers when making decisions for themselves compared to making decisions for others). These findings align with and extend prior research, showing that individuals often exhibit hyperaltruistic tendencies, valuing others' pain more than their own. In addition, M1 tDCS appears to moderate the influence of various contextual factors on decision-making tendencies. Our results also showed that hyperaltruistic behavior diminished with repeated decision-making tasks, potentially leading to egoistic behavior after repeated exposure to the same situations, offering important implications for both economic and social interactions in our society.

Disclosures: A. Dehghani: None. **D. Gantz:** None. **T.D. Wager:** None.

Poster

PSTR094: Neural Mechanisms: Choice in Reward

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR094.30/LL15

Topic: I.03. Decision Making

Support: NIH 5R01MH123713

Title: Prospective planning and credit assignment during model-based learning in a multi-step decision task

Authors: *L. J. H. RONDOT, E. D. BOORMAN;
Psychology, Learning & Decision Making lab - Ctr. for Mind and Brain - UC Davis, Davis, CA

Abstract: Credit assignment (CA) links outcomes back to the choices that caused them, enabling flexible, goal-directed behavior. However, real-life goals often require multi-step planning, where intermediate actions may not directly cause outcomes but are essential to achieving them. In such cases, updating the link between an outcome and its cause may also guide the next choice.

To examine the networks supporting CA and prospective planning, we used a modified two-step task (Daw, 2011). Participants (N=28) made sequential decisions in an MRI scanner: first-stage choices led probabilistically to one of two destinations, followed by second-stage choices leading to token rewards. In 10% of trials, tokens were reallocated between destinations, requiring participants to update their internal model.
We trained a classifier on neural patterns at each destination and tested whether the upcoming aimed-for destination could be decoded when the token is received. During common stable trials—when the plan was maintained—we decoded the aimed destination in the right hippocampus (HC; $t = 5.98$) and inferior temporal cortex ($t = 4.51$). In rare stable trials—when participants maintained their plan while avoiding the just-obtained outcome—decoding occurred in bilateral dlPFC (left: $t = 6.26$, right: $t = 3.56$; all $p < .01$ uncorr.). In common reallocation trials—when rewards were worse than expected and plans had to change—the new aimed destination was decoded in dlPFC ($t = 4.33$), dmPFC ($t = 3.48$), anterior HC ($t = 3.85$), and a midbrain region likely corresponding to VTA ($t = 3.75$). In rare reallocation trials—when unexpectedly better outcomes became the new goal—decoding appeared in putamen ($t = 3.9$), left HC ($t = 3.22$), and a region consistent with SNr ($t = 3.14$; all $p < .01$ uncorr.).
When the aimed destination matched the one that led to the token obtained (in common stable and rare reallocation), only HC represented both trial types, suggesting that other regions may signal plan maintenance vs. updating. dlPFC was consistently involved when the obtained destination should not be pursued, possibly reflecting a switch between CA and planning. Finally, the aimed destination could be decoded in the HC and brainstem following reallocation, supporting prospective planning. Ongoing work will test whether decoding strength predicts behavioral adaptation, and future directions include analysis to further dissociate and characterise CA from prospective planning.

Disclosures: L.J.H. Rondot: None. **E.D. Boorman:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.01/LL16

Topic: J.03. Anatomical Methods

Support: NIH Grant U01NS132353

Title: Inferring connectomes from RNA barcoding using graph neural networks

Authors: *D. KIENIGIEL, X. PITKOW;
Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Advances in viral barcoding techniques offer an unprecedented opportunity to scale throughput for obtaining connectomes. Current methods using rabies virus relies on retrograde spread of barcoded virions between neurons, and using barcodes found in multiple neurons to

infer connections between them. A major limitation of this method is that, due to multiple initial infections and polysynaptic spread, a significant fraction of sequenced barcodes can be explained by more than one barcoding source neuron. Using barcodes with ambiguous sources risks false positives, whereas discarding them leads to many false negatives.
To better understand the processes behind barcode infections, we developed mechanistic models of replication and transmission through synthetic connectomes and fit them to experimental sequencing data. These models highlight the effects of competition between barcodes, degradation, and consumption of limited intracellular resources. Our stochastic, discrete simulations scale linearly with the number of neurons and can simulate a week-long infection for 100K neurons in around 100s.
To infer synaptic connectivity that can account for measurable barcode co-occurrence, we developed graph neural networks (GNNs) to recover the known simulated connectomes from realistic measurements within our barcoding simulations. GNNs are general inference algorithms for graph-structured data, and are capable of explaining away some apparent ambiguities by propagating barcode information through potential synapses. By representing relationships in the sequencing data using hypergraphs, we are able to maintain the natural symmetries in the data, leverage both local and global information, and provide a flexible framework for increasingly complex barcoding strategies. We validate these GNNs through a wide range of network statistics and experimental and infection parameters. The outcomes on these simulated data are accurate even when tested out of distribution, an encouraging sign that it will generalize to in vivo data.
Knowing the ground truth, and by running simulations on a wide range of parameters, we are also able to optimize experimental parameters, such as finding what viral concentrations and incubation periods result in infections that are maximally informative about the connectome. This can drastically reduce the number of exploratory experiments that have to be performed, and provides a foundation for testing barcoding strategies before investing time and money on developing experiments and protocols.

Disclosures: D. Kienigiel: None. **X. Pitkow:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.02/LL17

Topic: J.03. Anatomical Methods

Support: NSF GRFP
National Institutes of Health BRAIN Initiative RF1-NS132046-01
Pershing Square Foundation Innovator Fund
Esther A. & Joseph Klingenstein Fund
Cold Spring Harbor Laboratory

Title: Selective expansion of motor cortical projections in the evolution of vocal novelty

Authors: *E. ISKO[1], C. HARPOLE[1], X. ZHENG[1], H. ZHAN[2], M. DAVIS[1], A. M. ZADOR[1], A. BANERJEE[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]MAPseq/BARseq Core Facility, Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Elucidating how neural architecture evolves to drive behavioral innovation remains a key challenge in neuroscience and evolutionary biology. In mammals, the neocortex is posited to play a role in facilitating rapid behavioral innovations. Although evolutionary models suggest that modifications in long-range connectivity can underlie such innovations, these hypotheses remain less tested quantitatively. To address this, we applied high-throughput barcode projection mapping to two closely related species with highly divergent behavior in a functionally relevant brain region.In this study, we focus on the divergent vocalizations in *Mus musculus* (lab mice) and *Scotinomys teguina* (singing mice). While most rodents, including lab and singing mice, produce short, ultrasonic vocalizations (USVs), singing mice display a novel vocalization of songs that are human-audible, stereotyped, and many seconds long. Additionally, manipulation and recording of the orofacial motor cortex (OMC) suggest a role of OMC in controlling song, leading us to hypothesize that modifications in the long-range projections of OMC neurons may lead to vocal innovations in the singing mice.To identify bulk projections of OMC neurons, we first performed viral tracing and serial two-photon tomography. We did not find any species differences in OMC projection targets, which included the contralateral cortex, striatum, thalamus, superior colliculus, periaqueductal grey (PAG), and others. To address whether there are quantitative differences within projection targets, we performed MAPseq, a high-throughput RNA barcoding technique, that resolves single-cell projection patterns. Sequencing of over 76,000 barcoded neurons revealed a specific and substantial expansion (~200%) of OMC projections to the auditory cortical region (AudR) and the midbrain periaqueductal gray (PAG), both implicated in vocal behaviors. Analysis of individual OMC neurons' projection motifs revealed preferential expansion of exclusive projections to AudR in the singing mice. Future experiments will integrate projection and transcriptomic data to explore whether transcriptional differences underlie the observed projection differences.Our results imply that selective expansion of ancestral motor cortical projections can underlie behavioral divergence over short evolutionary timescales, suggesting potential mechanisms for the evolution of enhanced cortical control over vocalizations. This approach of comparing recently-diverged species with large behavioral divergences can be readily generalized across other model clades to discover quantitative rules of neural circuit evolution.

Disclosures: E. Isko: None. **C. Harpole:** None. **X. Zheng:** None. **H. Zhan:** None. **M. Davis:** None. **A.M. Zador:** None. **A. Banerjee:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.03/LL18

Topic: J.03. Anatomical Methods

Title: Connectome-seq: Mapping Neural Connections and Molecular Identity with AAV-delivered RNA Barcodes and Parallel Single-nucleus + Single-synaptosome Sequencing

Authors: *B. ZHAO;
Univ. of Illinois Urbana Champaign, Urbana, IL

Abstract: Understanding neural circuit function requires knowledge of both synaptic connectivity and the molecular properties of connected neurons. We developed Connectome-seq, a novel sequencing-based approach that simultaneously maps synaptic connections and provides comprehensive transcriptomic profiling of connected cell pairs.
Connectome-seq uses engineered proteins to deliver unique RNA barcodes to pre- and postsynaptic compartments. These barcodes co-localize at synapses between connected neurons, enabling identification of synaptic partners through paired single-nucleus and single-synaptosome RNA sequencing. The method uses non-toxic AAV vectors for non-toxic, efficient delivery across multiple brain regions and can be applied to any circuit accessible to viral delivery.
We validated Connectome-seq in mouse pontocerebellar circuit, identifying established connections such as mossy fiber-granule cell pairs and revealing previously uncharacterized connectivity patterns. Critically, we discovered distinct transcriptomic signatures in connected neuron pairs, including specific marker genes enriched in Purkinje cells receiving pontine input, which we validated through independent viral tracing methods.
Beyond connectivity mapping, Connectome-seq revealed molecular determinants of circuit assembly by identifying candidate genes that may guide synaptic partner selection during development. We found that connected neurons exhibit complementary gene expression patterns, suggesting molecular determinants governing circuit formation.
This dual capability—simultaneous connectivity and molecular profiling—provides unprecedented insight into the relationship between gene expression and circuit organization. Connectome-seq offers the neuroscience community a powerful tool for dissecting circuit-specific molecular mechanisms underlying brain function, development, and disease states where connectivity patterns are altered.

Disclosures: B. Zhao: None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.04/LL19

Topic: J.03. Anatomical Methods

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NIH Grant U19NS123716
IARPA MICrONS D16PC0008
Robert Lourie award

Title: Massive Multiplexing of Spatially Resolved Single Neuron Projections with Axonal BARseq

Authors: *L. YUAN[1], X. CHEN[2], H. ZHAN[1], G. HENRY[1], A. M. ZADOR[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Allen Inst., Seattle, WA

Abstract: Neurons in the brain are highly heterogeneous and organized into complex circuits. Mapping their long-range projections at single-cell resolution remains a significant technical challenge due to the thin, intertwined nature of axons. To address this, we developed axonal BARseq, a high-throughput method for mapping long-range neural projections with single-cell resolution. As a proof of principle, we have mapped the long-range projections of >8,000 primary auditory cortex neurons from a single mouse. We identified major neuronal types based on their projection targets and axonal trajectories. The large dataset further allowed us to systematically characterize the projection diversity of intratelencephalic (IT) neurons, revealing distinctions in target regions, cortical layers, and focal versus broad projection patterns. Together, these results demonstrate that axonal BARseq is a powerful technique for studying the heterogeneity of neural projections.

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Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.05/LL20

Topic: J.03. Anatomical Methods

Support: NIH U01 CONNECTS (U01NS132161)
NIH DP2 (DP2MH132940)

Title: Barcoded connectomics in the mouse and macaque visual cortex reveals shared and distinct projection logic at cellular resolution

Authors: *M. MAJEED[1], H. QI[2], C. ELORETTE[3], S. Y. CHENG[4], A. ZHANG[1], A. OYAMA[1], J. ARIZA TORRES[1], Y. KOJIMA[4], H. ZHAN[5], S. KHEM[1], B. OUELLETTE[1], A. AYALA[1], E. LIANG[1], M. REDING[1], C. M. PAGAN[1], P. H. RUDEBECK[3], G. D. HORWITZ[4], J. M. KEBSCHULL[2], X. CHEN[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Johns Hopkins Univ., Baltimore, MD; [3]Icahn Sch. of Med. At Mount Sinai, New York, NY; [4]Univ. of Washington, Seattle, WA; [5]MAPseq/BARseq Core Facility, Cold Spring Harbor Lab., New York, NY

Abstract: Species-specific characteristics in connectivity are often accompanied by expansion and diversification of cell types and/or anatomical structures. For example, the macaque cortex

has sparser area-to-area connectivity compared to the mouse cortex, and this sparsification is accompanied by dramatic expansion of the number of cortical areas and diversification of cell types across layers. How species-specific features of connectivity arise at the single-neuron level, however, remains unclear. To address this question, here we applied BARseq to map single-cell projections in the macaque and mouse visual cortex to reveal how areas and cell types distinctively contribute to cortical connectivity to generate species-specific wiring characteristics. In BARseq, random RNA sequences, or barcodes, uniquely label thousands of neurons and fill up both somas and axons. Sequencing barcodes *in situ* in the somas and matching them to barcodes in axons reveals the projections of many neurons in parallel. Endogenous mRNAs can also be sequenced *in situ* to associate projections with transcriptomic identities at single-cell resolution. Using BARseq, we mapped the projections of 5,596 neurons in the mouse primary visual cortex (V1) and 500 neurons in macaque V1 at cellular resolution. Our data recapitulated known cell type- and laminar-specificity of projections and were consistent with the retinotopic organization of V1 projections to higher visual areas (HVAs). In both species, we found that more than half of all V1 neurons project to two or more HVAs, suggesting that the abundance of "broadcasting" neurons is a shared feature of corticocortical projections across species. Surprisingly, we found that soma locations, not coarse-level cell type identities, predominantly explained variations in corticocortical projection targets in the visual cortex. In contrast, coarse-level cell type identities determined the extent of axon collateralization, and a subset of fine-grained cell types further refined projection patterns. This organization was consistent across the two species, indicating conservation of single-cell wiring principles across species with distinct mesoscale connectivity. Our results provide a proof-of-principle for leveraging barcoded connectomics to understand circuit conservation and innovations across mammalian species.

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Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.06/LL21

Topic: J.03. Anatomical Methods

Support: 5R00MH121534-05, NIMH: Analysis of epigenetic and neuronal circuit changes in autism on the single-cell level

Title: Large-scale dissection of neuronal circuit development with inducible barcoded rabies virus

Authors: *D. VELMESHEV, Z. ZHANG;
Duke Univ., Durham, NC

Abstract: Brain development is a highly complex process that involves a multitude of cell types that are generated and specified during prenatal and early postnatal stages of development. Single-cell genomics and spatial transcriptomics methods greatly contributed to our understanding of this complexity, generating a taxonomy of molecularly defined cell types and gene programs that control neural lineage commitment and specification. However, brain development culminates in the formation and refinement of neural circuits that underline all brain functions. Understanding circuit formation at the resolution of individual cells and cell types is key to dissecting bases of normal behavior and mechanisms of brain disorders. However, progress in this area has been challenged by the lack of approaches that can dissect developmental circuit changes at scale and with single-cell resolution. To address this challenge, we developed a new inducible version of rabies virus, which we combined with molecular barcoding to produce highly complex viral libraries. We also designed a new experimental approach that allows labeling a large number of neurons and their presynaptic partners at a near whole-brain scale. We combine our approaches with single-cell genomics to map the dynamics of the developing mouse circuits at single-cell resolution. Our novel approach enables dissection of circuit development at scale and with single-cell resolution and provides novel granular insights into the principles of cortical circuit development and refinement.

Disclosures: D. Velmeshev: None. **Z. Zhang:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.07/LL22

Topic: J.03. Anatomical Methods

Support: NIH BRAIN 1R01NS111673
NIH TR01 5R01DC017876

Title: Investigating the logic of olfactory bulb-to-cortex projections at the level of single odorant-receptors/glomeruli circuit motifs

Authors: *Z. DAWOOD[1], C. SOITU[1], A. BECALICK[2], P. ZNAMENSKIY[2], A. M. ZADOR[1], A. KOULAKOV[1], D. ALBEANU[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]The Francis Crick Inst., London, United Kingdom

Abstract: Uncovering the rules ofsensory coding has been particularly challenging in modalities that lackconvenient reference dimensions of space and time, such as olfaction. Insuch cases, it is tempting to assume that sensory representations lackstructure and arise from randomly connected circuits—this has been theprevailing hypothesis for olfactory processing.We recently developed

high-throughput, DNA barcode sequencing-basedsingle-cell projection mapping methods that allow us to monitor the projectionpatterns of thousands of neurons in individual mice. These techniques,including *MAPseq* and *BARseq*, are capable of detecting structurein olfactory connectivity, if present. Using these methods, we discovered thatindividual mitral cells of the olfactory bulb (OB) tile the anterior-posterioraxis of the piriform cortex and project to extra-piriform regions, such as theanterior olfactory nucleus, cortical amygdala, and lateral entorhinal cortex,in coordination with their cortical targets, thereby forming triadic circuitmotifs. This body of work suggests that OB projections and the architecture ofthe olfactory cortex are indeed structured, and that models of olfactorycomputation need not rely on assumptions of random connectivity (Chen et al.,2022 *Cell*). Here, using *BARseq2.0*, we *investigate theinterplay between structure and randomness in the olfactory bulb output mitraland tufted cell (MTC) long-range projections as a function of their inputodorant receptor (OR) identity.* We inject barcoded Sindbis virus into the mitral andexternal plexiform layers along the A-P and dorsal-ventral axes of the OB. Insitu sequencing in 20 µm slices enables us to trace barcodes from MTC somatathrough apical dendrites to input glomeruli, identifying sister cells. OR genespecific probes label glomeruli by OR identity. We then perform MAPseq toreconstruct brain-wide projections of sister and non-sister MTCs and testwhether MTCs linked to a glomerulus target distinct cortical areas reproduciblyand non-randomly across animals.We analyze projection patterns by glomerulus, OR subfamily(Class I, II, TAAR), and ethological relevance. In pilot data, we barcoded3,912 MTCs and identified 41 sister sets. Our OR probes detected 506 unique ORsin situ across two animals, matching recent OR maps. Spatial analysis confirmeddorsal bias for Class I ORs and broader distribution for Class II. These dataenable the construction of glomerulus-to-cortex and OR-to-cortex projectionmatrices. Together, this approach bridges molecular, anatomical, and functionaldomains of the olfactory system and sheds light on how odor representations arestructured at the circuit level.

Disclosures: Z. Dawood: None. **C. Soitu:** None. **A. Becalick:** None. **P. Znamenskiy:** None. **A.M. Zador:** None. **A. Koulakov:** None. **D. Albeanu:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.08/MM1

Topic: J.03. Anatomical Methods

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NIH K08NS126573
NIH U01NS132353
Klingenstein-Simons Fund in Neuroscience

Title: High-complexity barcoded rabies virus for scalable circuit mapping using single-cell and single-nucleus sequencing

Authors: *M. E. URBANEK[1], D. SHIN[1], H. LARSON[1], A. MOUSSA[1,2], K. LEE[1], D. BAKER[1], E. STANDEN-BLOOM[1], S. RAMACHANDRAN[1], C. R. CADWELL[3], T. J. NOWAKOWSKI[1];
[1]Univ. of California San Francisco, San Francisco, CA; [3]Neurolog. Surgery, [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: The human cerebral cortex is comprised of a myriad of diverse neuronal cell types, which are organized into complex circuitry enabling advanced behaviors and cognition. Single cell genomics have revolutionized our understanding of these cell types; however, scalable technologies for examining connectivity with single cell resolution and molecular cell type annotation are lacking, and we are just beginning to understand how molecularly defined cell types may organize into functional circuits. We and others have recently developed barcoded rabies virus (RV) tools as a scalable, sequencing-based approach to map connectivity in mammalian brains. By incorporating a high-complexity molecular barcode into the RV backbone, such that each viral particle carries its own unique molecular identifier, we can map the synaptic inputs to many single cells in parallel. The number of neurons that can be studied at once is limited primarily by the complexity of the viral libraries that can be generated - both the total number of unique barcodes and the evenness of their distribution. We have developed an improved protocol for packaging G-deleted barcoded RV, which can be used for scalable connectivity mapping at the single cell level. Our protocol results in an approximately 100-fold increase in barcode complexity from previously published methods and leverages a bit-based barcode system with ≥6 hamming distance between each bit option, which enables straightforward computational deconvolution of unique barcode sequences. Additionally, we have devised a nuclear tethering mechanism for the barcodes (MCP-KASH-MS2), which enables simultaneous capture of both RV-encoded barcodes and cellular transcriptomes with snRNA-seq technology, making this technology compatible with flash frozen and adult human tissue. As a proof of principle, we applied barcoded RV to map the emergence of cell type-specific connectivity in organotypic slice cultures of developing human cortex. Across 9 slices, we identified a total of 12,036 unique barcodes, 2,762 of which originated in a single source cell and could be used to identify directional networks. Within these networks, we identified 4,331 unique connections (median=5 connections per source cell) and observed enrichment in connections across multiple transcriptomically defined subpopulations of neurons. By leveraging the power and throughput of single cell genomics for mapping synaptic connectivity, we chart a path forward for scalable circuit mapping of molecularly defined cell types.

Disclosures: M.E. Urbanek: None. **D. Shin:** None. **H. Larson:** None. **A. Moussa:** None. **K. Lee:** None. **D. Baker:** None. **E. Standen-Bloom:** None. **S. Ramachandran:** None. **C.R. Cadwell:** None. **T.J. Nowakowski:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional patent filed.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.09/MM2

Topic: J.03. Anatomical Methods

Support: NIH Grant RF1MH132596
NIH Grant U01NS132161
NIH Grant UM1NS132173

Title: Greatly increased efficiency of rabies virus production from DNA

Authors: H. A. SULLIVAN, ***I. R. WICKERSHAM**;
MIT, Cambridge, MA

Abstract: The first step of producing nonsegmented negative-strand (NNS) RNA viruses such as rabies virus is the so-called "rescue" of infectious virus particles from plasmid DNA, a highly inefficient process for these viruses which ordinarily never pass through a DNA phase in their life cycle. The inherent inefficiency of this unnatural act necessitates amplification of successfully-rescued clones by allowing them to reinfect producer cells and spread throughout the plate, in order to achieve titers high enough for typical neuroscientific uses. However, for barcoded rabies virus libraries, this inefficiency and compensatory amplification results in marked inhomogeneity of barcode distribution, with the virus preparation dominated by a small subset of overrepresented barcode clones. Multiplexed monosynaptic tracing, in which inputs to individual postsynaptic cells are separately identified, would benefit from considerably increasing the efficiency of virus rescue, to allow each starting cell to be infected by viral particles with barcodes that are unique to that cell among the starting cell population.
The state of the art in NNS virus rescue entails using self-cleaving ribozymes to create authentic ends of the viral genome (or antigenome) that can then be packaged by the viral nucleoprotein. Recently, a novel family of highly-efficient "twister" ribozymes was found to be widespread in eukaryotes and bacteria. Here we show that a twister ribozyme on the 3' end of the rabies viral antigenome increases rescue efficiency 37-fold over that of the most commonly-used 3' ribozyme (the standard hepatitis delta virus ribozyme) and 4.5-fold over that of the best previously-used 3' ribozyme (the "super cutter" hepatitis delta virus ribozyme). This increased efficiency should considerably increase the uniformity of barcode distribution in rabies virus preparations for multiplexed monosynaptic tracing applications.

Disclosures: H.A. Sullivan: None. **I.R. Wickersham:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.10/MM3

Topic: J.03. Anatomical Methods

Support: R34NS122050

Title: Surveying the connections of individual basolateral amygdala neurons to temporal cortex, frontal cortex, striatum and hypothalamus in macaques

Authors: *K. CRAWFORD[1], Z. R. ZEISLER[2], M. A. ELDRIDGE[3], E. A. MURRAY[4], P. H. RUDEBECK[2];
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Abstract: The amygdala, especially the basolateral group of nuclei (BLA), plays an essential role in cognition. In primates, a critical function of the BLA is to modulate the processing of emotionally salient stimuli, such as faces. Prior work has found that BLA sends projections to temporal lobe structures that process faces and we hypothesize that it is through these connections that BLA influences how such salient stimuli are processed. However, the structure and organization of these BLA connections at the level of single neurons to the temporal lobe remains unknown. One possibility is that individual BLA neurons branch and send collaterals to multiple regions of the temporal cortex as well as other parts of the frontal cortex, striatum, and hypothalamus. The recent application of multiplexed analysis of projections by sequencing (MAPseq) in macaques provides a method to study single neuron projections. To examine the anatomical projections of individual BLA neurons to the temporal and frontal lobes in rhesus macaques, we injected barcoded Sindbis virus into BLA and dissected regions of temporal and frontal cortex as well as the striatum and hypothalamus. In agreement with prior tract-tracing work, BLA neurons were most likely to project to the temporal polar periallocortex (TPPal), entorhinal cortex, and perirhinal cortex. BLA neurons were also highly likely to project to the hippocampus. Next, we looked at how BLA neurons collateralized to multiple anatomically distinct part of the temporal lobe. Here we found that neurons were more likely to project to multiple areas, with some neurons projecting to more than 4 distinct cortical areas. To look at how neurons collateralized, we focused on BLA neurons that targeted entorhinal, TPPal, and perirhinal cortex. Here we found that BLA neurons projecting to the entorhinal cortex were highly likely to also project to the hippocampus. This pattern was less prominent for TPPal and perirhinal cortex and instead, BLA neuron projections to these two areas were more likely to also project to parts of frontal cortex. Finally, we analyzed BLA projections that collateralized to temporal, frontal and hypothalamus. Here we found that BLA neurons that projected to frontal cortex and hypothalamus were highly likely to collateralize to and project to temporal cortex. Notably, BLA neurons projecting to frontal cortex were largely separate from those projecting to hypothalamus. Taken together our findings reveal that single BLA neurons exhibit distinct projections patterns within the temporal lobe and potentially begin to reveal how BLA has a wide-ranging influence on processing in temporal and frontal areas.

Disclosures: K. Crawford: None. **Z.R. Zeisler:** None. **M.A. Eldridge:** None. **E.A. Murray:** None. **P.H. Rudebeck:** None.

Poster

PSTR095: Connectomics I

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Topic: J.03. Anatomical Methods

Support: NIH Grant 1K99EY036489-01
NIH Grant 1U01NS136405-01
NIH Grant 5R01NS123912-04

Title: High-throughput retinotectal circuits mapping via TransA-MERFISH

Authors: *F. WANG[1], L. ZHANG[2], S. CHANG[3], C. LIU[4], X. DUAN[2];
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Abstract: Different visual features are initially decoded by various types of retinal ganglion cells (RGCs), which act as conduits from the eyes to the brain. With the help of mouse genetic markers and viral tools, mechanistic studies have mapped intra-retinal circuits, such as those responsible for direction selectivity, revealing the computational mechanisms underlying these circuits. In contrast, once RGC axons leave the retina and enter the brain, the types of cells they innervate and the computations they drive remain poorly understood. Clarifying the wiring diagrams from the retina to the brain, starting from defined RGC types, is crucial for achieving a comprehensive understanding of how visual information is processed in the brain. To achieve this goal, we integrated a suite of circuit tracing, spatial transcriptomics, genetics, and imaging toolsets to map the circuitry immediately downstream of genetically defined RGC types in the mouse superior colliculus (SC). First, we combined Multiplexed Error-robust Fluorescence In Situ Hybridization (MERFISH) with an anterograde trans- neuronal tracer (mWGA-mCherry, PMID: 35524141), termed TransA-MERFISH. This technique enables high-throughput neuron capturing in the adult brain while preserving the spatial distribution of individual neurons. By introducing mWGA-mCherry into genetically defined RGCs, such as alpha-RGCs or direction-selective RGCs, as starters, we then registered retino-recipient neurons onto the MERFISH-based SC neuron type atlas, respectively. This high-throughput, imaging-based method delineates comprehensive wiring diagrams from retinal cells to retinorecipient neurons in the SC with single-neuron-type resolution. Second, to validate circuit predictions from the TransA-MERFISH experiments, we initiated a new set of genetic, optogenetic, electrophysiological, and retrograde tracing experiments to investigate whether selective RGC- SC neuronal pairs are specified at retinotectal synapses. Third, building upon the molecules identified through spatial transcriptomics, such as Type II Cadherins (Cdh7, Cdh6, for example), we examined the roles of various cell-adhesive molecules in wiring the selective circuits identified via TransA-MERFISH. These findings help establish a molecularly and genetically tractable model in the mammalian brain, addressing a series of long-standing questions regarding the assembly and function of the early visual pathway.

Disclosures: F. Wang: None. **L. Zhang:** None. **S. Chang:** None. **C. Liu:** None. **X. Duan:** None.

Poster

PSTR095: Connectomics I

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Program #/Poster #: PSTR095.12/MM5

Topic: J.03. Anatomical Methods

Support: NIH Grant R01MH133181
NIH Grant U01NS132161
NIH Grant DP2MH132940

Title: Unraveling the single-cell logic of thalamocortical projections in marmoset using barcoded connectomics

Authors: A. FAN[1], H. QI[3], M. MAJEED[4], S. Y. CHENG[6], A. OYAMA[5], J. ARIZA TORRES[7], J. M. KEBSCHULL[9], J. A. BOURNE[2], ***X. CHEN**[8];
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Abstract: The marmoset medial pulvinar (PM) is a thalamic hub integrating information across association cortices and the frontal cortex. Previous tracing studies suggest topographic outputs from PM to both prefrontal (PFC) and visual association areas (VIS), but how this gradient arises at the single-cell remains unknown. Here, we applied Barcoded Anatomy Resolved by SEQuencing (BARseq) to map the transcriptomic identities and projections of over 20,000 neurons from the medial dorsal nuclei (MD) and the PM across four marmoset hemispheres. MD neurons primarily projected to PFC, whereas PM neurons projected to either PFC or VIS—but not both—at the single-cell level. These projection-defined neuron types were spatially intermingled across PM, suggesting that mesoscale topography reflects local shifts in cell type composition. While PFC-projecting neurons often targeted multiple PFC areas, VIS-projecting neurons typically targeted a single area. In situ sequencing linked projection types with distinct transcriptomic profiles in both PM and MD. A comparative analysis of mouse visual cortex projections neurons revealed a similar “topography-by-composition” organization, suggesting a conserved cross-species principle. These results reveal how thalamic projection gradients emerge from single-cell diversity, providing a framework for studying their evolutionary and developmental origins.

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Poster

PSTR095: Connectomics I

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Topic: J.03. Anatomical Methods

Support: NIH Grants R34NS132027
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Title: A framework for species-general barcoded connectomics

Authors: *H. QI[1], X. WAN[2], M. MAJEED[3], C. ELORETTE[4], A. FAN[5], L. WEI[7], M. ANANT[9], D. FALTINE-GONZALEZ[7], E. ZUERCHER[7], M. LOWMAN[7], C. WASHINGTON[7], J. A. BOURNE[6], G. D. HORWITZ[10], P. H. RUDEBECK[11], X. CHEN[12], J. M. KEBSCHULL[8]; [1]Johns Hopkins, Baltimore, MD; [2]McGill Univ., Montréal, QC, Canada; [3]Allen Inst. for Brain Sci., Seattle, WA; [4]Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Australian Regenerative Med. Inst., [6]Section on Cell. and Cognitive Neurodevelopment, NIMH, Bethesda, MD; [8]BME, [7]Johns Hopkins Univ., Baltimore, MD; [10]Physiol. and Biophysics, [9]Univ. of Washington, Seattle, WA; [11]Icahn Sch. of Med. At Mount Sinai, New York, NY; [12]Allen Inst., Seattle, WA

Abstract: Comparing brain connectivity across species can provide insights into the fundamental principles of neuronal organization. MAPseq (Multiplexed Analysis of Projections by Sequencing) enables high-throughput, rapid, and cost-effective mapping of brain-wide single-neuron projections, by translating anatomy into a sequencing problem. In this method, thousands of neurons are uniquely labeled with barcoded Sindbis virus. In each cell, barcodes are expressed as mRNAs and trafficked out into axon terminals by a carrier protein, where they can be detected by Illumina sequencing as quantitative readouts of each neurons' projections. MAPseq was originally developed in mice. Extending its use to other species remains technically challenging, due to species-specific differences in viral tropism and carrier protein efficiency. Here, we engineered a series of pseudotyped MAPseq viruses and screened these viruses for infection of Macaque, Marmoset, Zebra finch, Mouse, and Xenopus brains by BARseq2, identifying at least one pseudotype per species that supports robust neuronal infection. We further screened a series of synaptic proteins for efficient barcode trafficking in mice and zebra finches, and identified a species-general carrier protein. We finally apply our new method to map the connectivity of the zebra finch song system at single cell resolution. Taken together, our work extends MAPseq to a diverse range of vertebrates. It establishes a generalizable pipeline for adapting barcoded connectomics methods to any species of interest, and enables comparative connectomics.
Keywords: MAPseq, Connectomics, Barcodes, Neuron projections, songbird

Disclosures: H. Qi: None. **X. Wan:** None. **M. Majeed:** None. **C. Elorette:** None. **A. Fan:** None. **L. wei:** None. **M. Anant:** None. **D. Faltine-Gonzalez:** None. **E. Zuercher:** None. **M. Lowman:** None. **C. Washington:** None. **J.A. Bourne:** None. **G.D. Horwitz:** None. **P.H. Rudebeck:** None. **X. Chen:** None. **J.M. Kebschull:** None.

Poster

PSTR095: Connectomics I

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Program #/Poster #: PSTR095.14/MM7

Topic: J.03. Anatomical Methods

Support: NIH Grant 1RF1MH130468-01A1
NIH Grant 1U01NS136398-01
T32GM144273 (National Institute of General Medical Sciences)

Title: Genomic approaches for integrating gene expression and synaptic connectivity in the brain

Authors: *A. URKE[1], J. PINEDA[2], M. DOLAN[4], J. SILVERMAN[2], M. KIM[2], S. GARCIA[2], J. LUU[5], A. BUCKLEY[5], A. SVANBERGSSON[2], B. ZHAO[2], R. CHAI[6], W. MENEGAS[7], M. JIANG[8], K. LEVANDOWSKI[6], B. A. STEVENS[9], G. FENG[7], B. E. DEVERMAN[2], E. MACOSKO[3];
[1]Dept. of Biomed. Informatics, Harvard Univ., Boston, MA; [2]Broad Inst., Cambridge, MA; [3]Stanley Ctr. for Psychiatric Res., Broad Inst., Brookline, MA; [4]Broad Inst. of MIT and Harvard, Cambridge, MA; [5]Univ. of California San Francisco, San Francisco, CA; [6]MIT, Cambridge, MA; [7]MIT, Cambridge, MA, ; [8]MIT, cambridge, MA, ; [9]Harvard Med. Sch. Neurobio., Boston Children's Hosp., Milton, MA

Abstract: Mammalian brain function relies on synaptic connections between specialized cell types. However, we lack methods to systematically link transcriptomic identities and synaptic anatomy of single neurons at high-throughput. To this end, we developed Synapse-seq-a AAV-based technology that trafficks cell-identifying mRNA barcodes to pre- or postsynapses. While early applications focused on profiling long-range presynaptic cortical projections to brainwide targets (e.g. thalamus, striatum, medulla), we next established Synapse-seq as a novel, sequencing-based method for quantifying dendritic morphology.
Into the mouse hippocampus (HPF), we stereotactically injected our AAV-packaged postsynaptic constructs: 1) a PSD-95-targeting intrabody fused to green fluorescent protein and the RNA-binding protein PCP; 2) a 32bp mRNA barcode with mScarlet, a stability-enhancing pseudoknot, and PP7 stem loops. The layered HPF histology, in which excitatory soma are spatially segregated from postsynaptic processes, is ideal for benchmarking and applying our approach. First, we used in situ hybridization for mScarlet to confirm PP7 stem loop-dependent barcode trafficking to synaptic layers. Next, we performed Slide-seq on 22 coronal serial sections and targeted deep sequencing of barcoded viral transcripts (VTs). Using gene expression to annotate pyramidal (CA1, CA2, or CA3) or dentate granule (DG) beads, we showed that individual clusters of VT matches (“VT trees”) reproduced key cell-type-specific dendritic morphologies. Pyramidal VT trees exhibited longer apical and shorter basal distributions, while upper and lower DG distributions were unidirectionally opposed. Synapse-seq revealed a shift in anterior-posterior dendritic tree orientation from CA1 to CA3 and a cell type-specific increase in proximal apical density of CA1 dendrites. Finally, conducting an integrated comparison of

superficial-deep transcriptomic and morphological heterogeneity within/across all CA subfields, we observed prominent radial variation in CA1 apical dendrites (as quantified by Wasserstein distances) associated with postsynaptic differential gene expression signatures. Collectively, we establish Synapse-seq postsynaptic barcoding as a scalable platform for coupling dendritic morphology and transcriptional identity across large neuronal populations.

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Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.15/MM8

Topic: J.03. Anatomical Methods

Support: NIH UM1NS132253

Title: Precise Cutting and Collection of Whole Hemisphere Mouse Sections for Transmission Electron Microscopy

Authors: *X. LU[1], S. J. COOK[2], J. SCHARDT[1], M. M. TAKENO[3], A. L. BODOR[4], K. M. GRUVER[2], D. BRITTAIN[5], C. DEVINE[1], N. M. DA COSTA[4];
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Abstract: The primary barrier to scaling Electron Microscopy for mouse whole-brain imaging is not the imaging itself, but the physical sectioning of the brain—ensuring consistent, lossless serial cutting across centimeter-scale volumes remains a major technical hurdle. Overcoming this challenge is essential for enabling continuous, synapse-resolved imaging of an entire mouse brain. In this work, we focused on scaling up the Loop-based Automated Serial Sectioning Operation (LASSO) collection method, in which a loop is used to transfer the sections from the cutting boat to electron-transparent substrates. The LASSO collection method allows us to address several shortcomings of other methods, such as the ability to shuffle section order and reduce the probability of serial section loss. To accommodate LASSO for hemisphere collection, we designed a new wafer-like substrate that can hold hemisphere sections on electron-transparent film. We also optimized the loops and the boat for hemisphere-sized sections. To further automate the collection workflow, we added machine vision to LASSO and demonstrated the feasibility of cutting and collecting mouse hemisphere sections on to substrates. Once collected with LASSO, the whole hemisphere sections were imaged with Transmission Electron microscopy (TEM), a cost-effective, parallelizable, and non-destructive method to map the synaptic connectivity. TEM has proven its effectiveness in mapping the connectomes of various invertebrate systems as well as in handling multiple petascale datasets of the mammalian brain.

However, the technologies presented here also suitable for other nanoscale imaging technologies that depend on cutting and collecting sections at the whole mouse brain scale.

Disclosures: X. Lu: None. **S.J. Cook:** None. **J. Schardt:** None. **M.M. Takeno:** None. **A.L. Bodor:** None. **K.M. Gruver:** None. **D. Brittain:** None. **C. Devine:** None. **N.M. da Costa:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR095.16/MM9

Topic: J.03. Anatomical Methods

Support: NIH Grant MH117820
NIH Grant UG3MH126864
NIH Grant UM1NS132207

Title: Combined lightsheet microscopy pipelines for multiscale mapping of mammalian brain connectivity

Authors: *K. TAKASAKI[1], E. E. TURSCHAK[1], S. J. COOK[1], K. VILLALOBOS[1], E. GUADARRAMA[1], R. TORRES[1], W.-Q. YU[1], C. LAUGHLAND[1], O. GLIKO[1], A. HELLEVIK[1], S. CHATTERJEE[1], E. PERLMAN[1], F. C. COLLMAN[1], N. M. DA COSTA[1], A. GLASER[2], R. REID[1];
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Abstract: Understanding the role of connectivity in determining mammalian brain function is a major goal of neuroscience; however, the complex anatomical organization of large brains on spatial scales spanning many orders of magnitude, from nm-scale synapses to cm-scale fiber tracts, poses a significant challenge. To address this challenge, various technologies have been developed for connectivity mapping at different spatial scales. In small volumes, dense anatomical reconstructions by serial-section electron microscopy resolve connections between neurons on the level of individual synapses, while on the whole-brain level, imaging methods such as DTI, OCT, and x-ray tomography allow brain-wide mapping of fiber tracts and areal projections. Recent progress in tissue clearing and expansion, and in volumetric lightsheet microscopy has advanced fluorescence imaging toward both extremes of ultrastructural resolution and whole-brain imaging capabilities, presenting the opportunity for novel studies of connectivity across spatial scales with light microscopy. Here, we describe a combined data acquisition approach encompassing two lightsheet microscopy pipelines used in concert to relate mammalian brain connectivity at spatial scales across several orders of magnitude. In brief, a large-format, high-throughput imaging pipeline for axonal connectomics enabled by expansion-assisted selective plane illumination microscopy (ExA-SPIM; Glaser, Chandrashekar, et al.) generates dense projection maps of individual axons across large tissue volumes, which can then

be combined in regions of interest with EM-like anatomical reconstruction (light-microscopy-based connectomics, LICONN; Tavakoli et al.) enabled by ultrastructural resolution with iterative tissue expansion and confocal Bessel beam lightsheet microscopy. We report the results of ExA-SPIM imaging of singly expanded (3-4x) serial sections with immunolabeled neurofilament proteins, yielding fluorescence imaging of densely stained axons with near isotropic 250 nm effective resolution and enabling computational tracing of individual axons over centimeters of tissue. With the addition of a pan-protein stain with iterative expansion (16x) for LICONN, we further demonstrate targeted optical imaging with 13 nm resolution with a custom lightsheet microscope combining high-NA imaging with scanning Bessel beam illumination and virtual confocal slit detection. We seek to apply multiscale data to long-range axonal and local circuit connectomics toward the overall goal of mapping individual axons from their areas and cell types of origin, through the white matter, to their distant targets.

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Poster

PSTR095: Connectomics I

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Program #/Poster #: PSTR095.17/MM10

Topic: J.03. Anatomical Methods

Support: NIH BRAIN CONNECTS UM1NS132253

Title: Whole-mouse hemisphere staining for electron microscopy

Authors: J. SCHARDT, **M. M. TAKENO**, A. L. BODOR, S. J. COOK, K. M. GRUVER, X. LU, D. BRITTAIN, *N. M. DA COSTA;
Allen Inst., Seattle, WA

Abstract: Whole mouse brain electron microscopy (EM) requires staining protocols that preserve ultrastructure at nanometer resolution across centimeter-scale tissue volumes. Building on foundational methods developed by Mikula and Denk , recent advances have improved the reliability and reproducibility of whole-brain staining (Song, Feng, and Helmstaedter 2023; Lu et al. 2023). Here, we present a variant of the protocol introduced by Song et al., substituting the original resin with LX-112 (an Epon 812 replacement; Ladd Research) and reducing the number of osmium rounds to facilitate sectioning and reduce sample artifacts. We applied this protocol to mouse whole hemispheres, from which ultrathin (80 to 120 nm thick) whole hemisphere sections have been cut and imaged at nanometer resolution to evaluate sample quality. Although whole-brain protocols have typically been validated using scanning electron microscopy (SEM), we demonstrate that this approach also yields high-quality results with transmission electron

microscopy (TEM), despite the distinct contrast mechanisms between the two modalities. The protocol maintained high quality ultrastructural integrity without detectable cracks, albeit with reduced contrast, as seen with both EM and MicroCT. This protocol offers a reproducible and approach for preparing whole-mouse brains for high-resolution EM across multiple imaging platforms.References:
Lu X, Wu Y, Schalek RL, Meirovitch Y, Berger DR, Lichtman JW. A Scalable Staining Strategy for Whole-Brain Connectomics. bioRxiv [Preprint]. 2023 Sep 27:2023.09.26.558265. doi: 10.1101/2023.09.26.558265. PMID: 37808722; PMCID: PMC10557665.
Mikula S, Binding J, Denk W. Staining and embedding the whole mouse brain for electron microscopy. Nat Methods. 2012 Dec;9(12):1198-201. doi: 10.1038/nmeth.2213. Epub 2012 Oct 21. PMID: 23085613.
Song K, Feng Z, Helmstaedter M. High-contrast en bloc staining of mouse whole-brain and human brain samples for EM-based connectomics. Nat Methods. 2023 Jun;20(6):836-840. doi: 10.1038/s41592-023-01866-3. Epub 2023 May 8. PMID: 37156843; PMCID: PMC10250191.

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Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.18/MM11

Topic: J.03. Anatomical Methods

Support: UM1NS132253

Title: Toward high-throughput automated nanoscale TEM imaging of whole mouse hemisphere sections

Authors: *K. M. GRUVER, X. LU, D. BRITTAIN, C. DEVINE, A. L. BODOR, J. SCHARDT, S. J. COOK, M. M. TAKENO, R. TORRES, R. REID, F. C. COLLMAN, N. M. DA COSTA;
Allen Inst., Seattle, WA

Abstract: The synaptic relationships between cells underlie diverse brain circuits. Transmission electron microscopy (TEM) can be used to map synaptic connections with incredible detail, including in serial-section volumetric sample preparations. While TEM is typically used to resolve local connections between cells, whole hemisphere imaging of mouse brains using TEM can also enable identification of long-range connections across brain regions. To explore the feasibility of whole hemisphere TEM imaging, we performed automated imaging of serial whole mouse hemisphere sections at nanometer resolution. We redesigned key TEM objective lens hardware to facilitate large sample imaging of both sagittal and coronal hemisphere mouse sections. Our automated imaging software enabled the acquisition of multiple terabytes of data

per hemisphere section at multiple resolutions. This work lays the foundation to scale TEM imaging to centimeter scale volumes and evaluate the feasibility of imaging whole hemisphere samples at the resolution of synapses.

Disclosures: K.M. Gruver: None. **X. Lu:** None. **D. Brittain:** None. **C. Devine:** None. **A.L. Bodor:** None. **J. Schardt:** None. **S.J. Cook:** None. **M.M. Takeno:** None. **R. Torres:** None. **R. Reid:** None. **F.C. Collman:** None. **N.M. da Costa:** None.

Poster

PSTR095: Connectomics I

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Program #/Poster #: PSTR095.19/MM12

Topic: J.03. Anatomical Methods

Title: Combining protein barcoding, highly multiplexed expansion microscopy, and automatic proofreading with barcodes to enable scalable optical connectomes

Authors: S. PARK[1,2], A. SHERIDAN[2], J. M. MICHALSKA[2], J. LYUDCHIK[2], E. JARVIS[2], B. AN[3], S. CHAN[2], M. WU[2], H. DAMSTRA[2], A. MEERAN[2], C. WANG[2], J. CHEN[2], J. LEE[2], J. AXUP[2], C. MAGNO[2], D. LEIBLE[3], K. LEEPER[2], S. TRUCKENBRODT[2], J. WINNUBST[2], J. KORNFELD[3], E. S. BOYDEN[3,4], S. RODRIQUES[1], ***A. PAYNE**[2];
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Abstract: Here we introduce PRISM (Photo-connectomic Reconstruction by Iterative Staining with Molecular annotations), which integrates combinatorial AAV infection of cell-filling protein barcodes, highly multiplexed 6-fold expansion microscopy, and automated barcode-guided segmentation to deliver morphological reconstructions with rich molecular annotations. Applying PRISM to a 13 million cubic micron volume of mouse hippocampus CA3, we obtained robust readout of 18 protein barcode channels and multiple synaptic markers with reliable trafficking of protein barcodes into distal processes. We used molecular annotations to map excitatory and inhibitory synapse distributions and putative connections between pre- and post-synaptic barcoded neurons in the CA3 volume, demonstrating extensibility of PRISM for investigating neuronal circuit architecture. We show barcodes can be used to improve automated segmentation at reduced costs, and to facilitate automated proofreading by reconnecting falsely split segments. Taken together, we envision PRISM as a scalable path to integrate high resolution morphology with molecular information, opening new avenues for high-throughput circuit-targeted investigations and therapeutics.

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Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR095.20/MM13

Topic: J.03. Anatomical Methods

Support: NIH Grant UG3MH126864
NIH Grant UM1NS132207
NIH Grant MH117820

Title: Streamlining Axon Labeling and Segmentation in Large Volumes of Human Brain Tissue

Authors: *E. GUADARRAMA[1,2], E. TURSCHAK[2], A. HELLEVIK[2], K. VILLALOBOS[2], K. TAKASAKI[2], R. TORRES[2], O. GLIKO[2], W.-Q. YU[2], S. CHATTERJEE[2], C. LAUGHLAND[2], S. J. COOK[3], R. REID[3];
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Abstract: Visualizing white matter organization at single-axon resolution is critical for generating a comprehensive wiring diagram of the human brain. Such a map would permit comparison of neural circuits and axons in healthy and disease states. However, centimeter-scale projection mapping of individual axons is difficult, as the density of white matter and high levels of fixation often used in archived human tissue obstruct antibody penetration and limit the ability to visualize individual structures at high resolution. To overcome these challenges, we developed an expandable hydrogel-based histological pipeline for isotropic tissue expansion, paired with Expansion-Assisted Selective Plane Illumination microscopy (ExA-SPIM) for sub-micron resolution imaging. We processed 500µm-thick tissue from different white matter regions of the brain including the corpus callosum, cingulum, and centrum semiovale, to compare the variety of organizations found in these white matter-rich regions. We compared different tissue preparation methods such as using fresh surgical tissue, alongside our current flash-frozen fixed tissue, to evaluate differences in structural integrity and labeling quality in order to improve the reliability and scalability of our pipeline.To effectively map large regions of the human brain we need bright and specific axonal staining. We are also exploring the use of NHS-esters to broadly label all proteins. Currently, we robustly label axons using an antibody against neurofilament heavy chain proteins (NEFH), but this strategy does not label the soma or dendrites, limiting our ability to trace axons from the cell body through their full trajectory. To evaluate whether NHS ester labeling alone can reliably replace neurofilament staining for unambiguous axonal identification, we co-labelled axons with neurofilament antibodies and NHS-esters, revealing a high degree of colocalization. Given the broad labeling of NHS staining, our current strategy is to train a classification model for the accurate segmentation of axons from surrounding structures. NHS-

only labeling has the potential to streamline staining protocols and reduce costs, and in combination with improved tissue processing, enhance scalability towards whole brain connectomics.

Disclosures: E. Guadarrama: None. **E. Turschak:** None. **A. Hellevik:** None. **K. Villalobos:** None. **K. Takasaki:** None. **R. Torres:** None. **O. Gliko:** None. **W. Yu:** None. **S. Chatterjee:** None. **C. Laughland:** None. **S.J. Cook:** None. **R. Reid:** None.

Poster

PSTR095: Connectomics I

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Program #/Poster #: PSTR095.21/MM14

Topic: J.03. Anatomical Methods

Support: Allen Institute Proteomics Initiative
NIH Grant UG3MH126864
NIH Grant UM1NS132207

Title: Progress toward high-throughput expansion and light microscopy-based connectomics

Authors: *K. L. VILLALOBOS[1], E. E. TURSCHAK[3], E. GUADARRAMA[4], K. TAKASAKI[1], N. M. DA COSTA[3], R. REID[2], S. J. COOK[2];
[2]Neural Coding, [1]Allen Inst. for Brain Sci., Seattle, WA; [3]Allen Inst. For Brain Sci., Seattle, WA; [4]Allen Inst., Seattle, WA

Abstract: Developing a comprehensive map of the brain is essential for advancing our knowledge of normal brain structure and identifying circuit alterations in neurodegenerative diseases. However, large-scale comprehensive neural circuit mapping requires imaging techniques that balance resolution, throughput, and cost-effectiveness for nanoscale connectomics studies. The light microscopy-based connectomics (LICONN) approach uses iterative hydrogel-based expansion and pan-protein fluorescent labeling to generate high-resolution imaging datasets that approach electron microscopy (EM) resolution, allowing for detailed neural circuit visualization. While proven using confocal microscopy, this modality lacks the imaging speed necessary for large-scale analysis, creating a bottleneck for comprehensive circuit reconstruction. Therefore, to achieve the necessary throughput while maintaining high image quality, we used a custom Inverted Selective Plane Illumination Microscopy (ISPIM) system that employs a bessel beam that uses confocal detection principles to reject out-of-focus fluorescence, achieving near confocal-level resolution at lightsheet speeds. We assessed the feasibility of dense anatomical reconstruction using LICONN lightsheet data across multiple systems. Mouse brain tissue processed with LICONN was imaged using both the custom Bessel-beam ISPIM system and a commercial Zeiss Lightsheet Z.1 microscope to generate high-resolution volumes compatible with nanoscale EM connectomics workflows. In collaboration with computational partners at Zetta and Google, we are applying segmentation

algorithms to identify optimal approaches for dense reconstruction of neurons and synapses from LICONN data. Preliminary findings demonstrate successful generation of lightsheet volumes suitable for automated segmentation analysis. This approach has the potential to bridge traditional fluorescence microscopy and EM-based connectomics, suggesting future applications for comprehensive connectivity analysis across multiple brain regions and representing a significant advance toward scalable connectomics studies.

Disclosures: K.L. Villalobos: None. **E.E. Turschak:** None. **E. Guadarrama:** None. **K. Takasaki:** None. **N.M. da Costa:** None. **R. Reid:** None. **S.J. Cook:** None.

Poster

PSTR095: Connectomics I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR095.22/MM15

Topic: J.03. Anatomical Methods

Support: NIH UM1NS132253

Title: New hardware and software solutions for centimeter-scale nanoconnectomics

Authors: *S. J. COOK[1], X. LU[1], A. GUTHRIE[2], K. M. GRUVER[1], D. BRITTAIN[1], J. SCHARDT[4], M. M. TAKENO[5], R. REID[3], N. M. DA COSTA[6];
[2]Scientific Instrumentation and Process Engin., [3]Neural Coding, [1]Allen Inst. for Brain Sci., Seattle, WA; [4]Neural Coding, Allen Inst., Seattle, WA; [5]Neural Coding, [6]Allen Inst. For Brain Sci., Seattle, WA

Abstract: Interest in generating complete invertebrate connectomes and partial mammalian connectomes is increasing, despite the technical challenges involved. To date, nanoscale connectomes generated from serial section EM volumes have relied on a combination of expert sample preparation techniques and specialized hardware engineering. As the field progresses toward centimeter-scale brain mapping of complete mammalian connectomes, the need for adaptable, automated hardware and software solutions becomes critical. We developed an integrated suite of tools designed around three core principles: affordability, modularity, and straightforward implementation, addressing the technical constraints of existing commercial solutions for volume electron microscopy while making connectomic methods more accessible to research laboratories. We have written open-source software to control these devices robustly and modularly, with the ability to perform quality control not possible with commercial devices. Drawing from established research demonstrating that linear stage and piezo actuator combinations can cut high-fidelity serial EM sections (iTome, https://www.biorxiv.org/content/10.1101/2024.07.02.601671v1), we engineered a custom ultramicrotome system that can consistently cut sections with millimeter-scale lateral dimensions down to 15 nanometer thickness and entire mouse hemispheres at centimeter scale to 80 nanometer thickness. We also modified a loop-based robotic mechanism (LASSO) that can

automatically transfer sections onto imaging substrates, accommodating the stringent positioning and handling requirements for TEM imaging. By physically modifying TEM sample access, we can image montages of these whole hemisphere sections at 4nm pixel resolution. These technological advances establish a versatile platform for connectomic research that supports neural circuit mapping across multiple scales, and by prioritizing accessibility and modularity in our design approach, we aim to broaden the adoption of connectomic techniques throughout the neuroscience research community, facilitating more widespread investigation of neural wiring diagrams.

Disclosures: S.J. Cook: None. **X. Lu:** None. **A. Guthrie:** None. **K.M. Gruver:** None. **D. Brittain:** None. **J. Schardt:** None. **M.M. Takeno:** None. **R. Reid:** None. **N.M. da Costa:** None.

Poster

PSTR096: Probe Design and Engineering I

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.01/MM16

Topic: J.04. Physiological Methods

Support: UM1 MH136462-01

Title: Directed evolution of dopamine biosensors via a high-throughput robotic screening platform

Authors: *L. LEE[1], N. TJAHJONO[2,1], K. PODGORSKI[3], M. Z. LIN[4], K. SVOBODA[3], L. TIAN[1];
[1]Max Planck Florida Inst. for Neurosci., Jupiter, FL; [2]Univ. of California, Davis, Davis, CA; [3]Allen Inst. for Neural Dynamics, Seattle, WA; [4]Neurobio., Stanford, Stanford, CA

Abstract: The release of chemical messengers is the fundamental process underlying synaptic communication in the brain. Dopamine is one such messenger, a neuromodulator that plays a crucial role in various brain functions such as decision-making, reinforcement learning, and motor control. Despite its important physiological role, there are few options for measuring neurochemical release in vivo with high spatiotemporal resolution and cell-type specificity. To address this need, our lab developed the genetically encoded fluorescent dopamine biosensor, dLight. This indicator increases in brightness upon binding to its chemical target, enabling real-time detection of dopamine. Typically, dLight and other similar sensors are developed through a semi-rational approach, employing site-saturated mutagenesis of a few residues predicted to impact sensor performance. However, existing screening methods are either able to test only a highly limited number of candidates or face challenges when translating to in vivo applications, making biosensor development a slow and expensive process. Recently, certain robotic screening approaches have shown improved throughput over traditional methods. Building on this direction, we have developed a high-throughput biosensor screening platform capable of rapidly

screening tens of thousands of sensor variants in mammalian cells. This method improves throughput over previous mammalian cell screening methods by orders of magnitude. We leveraged the improved throughput of this platform to pursue two protein engineering strategies to expand in vivo applications of dopamine sensors. Using a directed evolution approach, we developed an improved version of dLight with significantly improved dynamic range and similar molecular specificity. We also used a deep mutational scanning library to characterize and sequence hundreds of single-mutation variants. From this dataset, we identified several regions of dLight that are rich in beneficial mutations. In the future, we can leverage such sequence datasets, together with their corresponding biophysical characteristics, to pursue sensor screening and engineering in silico. Overall, this platform represents a reproducible, high-throughput method for the rapid development and optimization of genetically encoded biosensors, reducing barriers to neuroscience tool development.

Disclosures: L. Lee: None. **N. Tjahjono:** None. **K. Podgorski:** None. **M.Z. Lin:** None. **K. Svoboda:** None. **L. Tian:** None.

Poster

PSTR096: Probe Design and Engineering I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.02/MM17

Topic: J.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Optimization of genetically encoded functional indicators for in vivo imaging: GENIE Project Team updates

Authors: *G. C. TURNER[1], A. AGGARWAL[3], B. J. ARTHUR[4], H. R. FARRANTS[5], J. P. HASSEMAN[5], V. JAYARAMAN[5], J. N. KOBERSTEIN[1], W. KORFF[6], A. NEGREAN[7], A. PALUTLA[1], K. PODGORSKI[8], B. G. POWER[2], D. REEP[1], E. R. SCHREITER[5], A. STUART[9], A. G. TEBO[10], A. TSANG[1], G. TSEGAYE[11], A. WARING[1], J. ZHENG[6];
[2]GENIE Project Team, [1]Janelia Res. Campus, Ashburn, VA; [3]Univ. of Calgary Cumming Sch. of Med., Calgary, AB, Canada; [4]Howard Hughes Med. Inst., ashburn, VA; [5]Janelia Res. Campus, HHMI, Ashburn, VA; [6]HHMI Janelia Res. Campus, Ashburn, VA; [7]Neural Dynamics, Allen Inst., Seattle, WA; [8]Allen Inst. for Neural Dynamics, Seattle, WA; [9]Johns Hopkins / HHMI Janelia, Ashburn, VA; [10]HHMI – Janelia Res. Campus, Ashburn, VA; [11]HHMI, Ashburn, VA

Abstract: GENIE is a Janelia Project Team focused on the development of genetically encoded sensors for cellular activity. We utilize systematic mutagenesis and high-throughput screening in primary culture to optimize sensor performance. Our pipeline spans biochemical characterization to in vivo validation in mice, zebrafish, and flies using functional imaging. We will present the latest results of optimizing genetically encoded indicators for calcium and neurotransmitter release.

SCaMP: We developed a new red calcium indicator based on mScarlet. Initial variants were generated via directed evolution and screened in bacterial lysates. Subsequent rounds of site-directed mutagenesis and primary neuron screening yielded single-site variants with ~5-fold higher signal:noise than jRGECO and jRCaMP.
We then took 61 single-site variants with the best performance, and evaluated the entire set of their pairwise combinations. The best combination showed a 4-fold improvement in signal-to-noise over the top single mutant. We will present comparisons of SCaMP to existing red calcium indicators in terms of signal:noise, baseline fluorescence (F_0), kinetics, and photostability. Top variants are currently being tested in vivo in mouse visual cortex, the Drosophila head direction circuit, and larval zebrafish. SCaMP's red-shifted spectrum and enhanced performance make it well suited for deep-tissue imaging and two-color imaging alongside GCaMP, and it is compatible with blue-light optogenetics.
iGluSnFR4: We developed improved variants of this sensor of glutamate release via primary neuron screening with a focus on kinetics and the detection of single release events1. After further evaluating performance in vivo, we identified two high-performing variants with increased signal:noise and rapid activation kinetics (< 2 ms), but differing deactivation time constants. iGluSnFR4s ($\tau off \approx 153$ ms) is well-suited for imaging large populations of synapses, while iGluSnFR4f ($\tau off \approx 26$ ms) enables resolution of high-frequency synaptic activity.
WHaloCaMP2: Is a hybrid chemigenetic calcium indicator composed of a genetically encoded calcium-sensing domain and a HaloTag-attached synthetic dye. This sensor enables imaging at near-infrared wavelengths, depending on the choice of the synthetic dye from the JF family. Our screening pipeline identified variants with 2-5x increased sensitivity to single action potentials, whose performance will now be tested in vivo.
1 Aggarwal, A. et al. bioRxiv 2025.03.20.643984 (2025)

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Poster

PSTR096: Probe Design and Engineering I

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Topic: J.04. Physiological Methods

Support: NIH U01NS139877-01
NIH U01NS131914-02
NIH 1DP2EB030992-01

Title: Label-free Optical Detection of Cellular Activity Utilizing Quantum Statistics in Monolayer Two-dimensional Materials

Authors: *F. SUN[1], Y. REN[2], M. WILSON[1], C. CRAIN[1], M. RAMEZANI[1], D. KUZUM[1], E. CUBUKCU[1,2];
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Abstract: Monitoring the electrical activity of excitable cells, in particular action potentials, is fundamental to understanding both individual and systematic cell behavior. Recent advances in quantum-confined two-dimensional (2D) materials have enabled label-free, all-optical recording of electrical activity with high spatiotemporal resolution. These materials exhibit a strong excitonic response, and their photoluminescence (PL) is sensitive to localized electric fields via exciton-trion conversion. This allows for the detection of action potentials via voltage-induced PL modulation. In particular, monolayer MoS_2 serves as a bias-free, tetherless optical voltage sensor due to its intrinsic background electron density, which is primarily contributed by sulfur vacancies. This enables it to detect sub-millivolt potential fluctuations in physiological environments without the need for genetic modification or chemical labeling of cells or tissues. In addition, the semiconducting property of MoS_2 allows for simultaneous electrical recording, enabling hybrid optical-electrical validation in real time. In this work, we present MoS_2-based optical sensors for minimally invasive, label-free detection of action potentials intracellular and extracellularly. We demonstrate sensing of spikes with kHz speeds, compatible with multiple optical microscopy setups including one photon and two photon microscopy. The platform provides a promising foundation for high-resolution voltage imaging in complex biological and neurophysiological settings.

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Poster

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Topic: J.04. Physiological Methods

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New Cornerstone Science Foundation through the New Cornerstone Investigator Program
Peking-Tsinghua Center for Life Sciences and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences
NIH BRAIN Initiative 1U01NS120824
Beijing Municipal Science & Technology Commission Z220009

Title: Development of a genetically encoded fluorescence lifetime-based dopamine sensor

Authors: *Y. YANG, Y. ZHENG, Z. WANG, Y. LI;
Peking Univ., Beijing, China

Abstract: Dopamine is a key neurotransmitter involved in many physiological functions and pathological processes. To understand the complex functions of dopamine in various conditions, it is important to precisely and quantitatively measure its dynamics. Building on the GRAB (GPCR Activation Based) strategy, our lab has previously developed a series of green and red fluorescent dopamine sensors. However, these intensity-based sensors are limited in their capacity for quantitative imaging due to susceptibility to intensity-related artifacts. Fluorescence lifetime, an intrinsic property of fluorophores that is independent of fluorescence intensity, provides a powerful alternative for quantitative imaging by eliminating intensity-based variability while maintaining high spatial and temporal resolution. Leveraging this property, we developed a fluorescence lifetime-based dopamine sensor that is capable of detecting dopamine dynamics in cultured cell lines, cultured primary neurons, and acute brain slices. Further optimization and characterization of this sensor aim to enhance its quantitative capability in vivo. The lifetime-based dopamine sensors represent a valuable addition to the neuroimaging toolkit, offering new opportunities to investigate the complex dopaminergic system and its involvement in both physiological and pathological contexts.

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Poster

PSTR096: Probe Design and Engineering I

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.05/NN2

Topic: J.04. Physiological Methods

Support: NIH grant NS123658

Title: Campari3 - a high-sensitivity ratiometric calcium activity integrator

Authors: A. DAS[1], M. MUSA[2], D. R. MARGEVICIUS[1], J. P. ICARDI[1], S. BHATIA[1], M. BOISVERT[3], R. CAMPBELL[4], M.-E. PAQUET[5], ***H. DANA**[1];
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Abstract: CaMPARI is a calcium- and light-dependent fluorescent activity integrator that is used to mark active neurons *in vitro* and *in vivo* through a process known as photoconversion. Photoconverted CaMPARI changes its emission fluorescence from green to red. This change is irreversible at the protein level; however, natural cellular protein turnover causes the red signal to largely disappear within seven days. Therefore, CaMPARI provides experimenters with a tool to highlight activity-dependent patterns that survive a relatively long times and also can be used

for repeated recordings. Since the development of CaMPARI ten years ago, a second-generation sensor, CaMPARI2, has been introduced, offering enhanced brightness and improved contrast between the green and red states. However, a recent work from our lab showed a mismatch between the prediction of the CaMPARI2 *in vitro* development assay to its *in vivo* performance. Here, we present the outcome of a large-scale *in vitro-in vivo* optimization project to develop a new generation of the CaMPARI sensor, CaMPARI3. We generated and screened the same CaMPARI constructs in various *in vitro* and *in vivo* assays. Our findings show that photoconversion rates of the same variants *in vitro* and *in vivo* do not correlate. Rather, the peak fluorescence change *in vitro* may better predict the rate of photoconversion *in vivo*. We also present the new CaMPARI3, which exhibits a 5- to 10-fold improvement in photoconversion rate compared to the previous generations and enables conducting recording experiments using significantly shorter illumination times. In addition, CaMPARI3 has a significantly improved capability to record dynamic changes in neuronal activity, similar to standard calcium sensors. These enhanced capabilities make CaMPARI3 a high-sensitivity version of the CaMPARI sensor and will expand the possible experimental applications to record single-cell resolution brain activity under various experimental conditions.

Disclosures: A. Das: None. **M. Musa:** None. **D.R. Margevicius:** None. **J.P. Icardi:** None. **S. Bhatia:** None. **M. Boisvert:** None. **R. Campbell:** None. **M. Paquet:** None. **H. Dana:** None.

Poster

PSTR096: Probe Design and Engineering I

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Topic: J.04. Physiological Methods

Support: NIH Grant UM1MH136462
Allen Institute

Title: A platform for benchmarking genetically encoded neuromodulator indicators in vivo

Authors: B. J. MACLENNAN[1], B. WYNALDA[2], S. NARAYAN[2], K. SVOBODA[1], K. PODGORSKI[1], *K. M. HAGIHARA[1];
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Abstract: Neuromodulators (NMs) have been implicated in a variety of biological and behavioral functions. The development of fluorescent protein indicators for NMs has exploded in recent years, enabling temporally precise optical measurements of NM dynamics on the membranes of targeted cell types. However, there is a need for systematic and quantitative evaluation of indicator performance *in vivo*, especially as multiple indicators exist for the same NM. *In vitro* assays do not translate to *in vivo* performance because of differences in chemical environment, tissue architecture, and ligand concentration dynamics. Currently, a lack of systematic benchmarking makes it challenging for researchers to select the appropriate indicator

for specific applications. Here, we present a platform to benchmark indicator performance *in vivo*. To achieve consistent expression levels, indicators for norepinephrine (NE), serotonin (5-HT), acetylcholine (Ach), dopamine (DA), and Histamine (His) were expressed using standardized adeno-associated viruses (AAVs). Optical fiber-based photostimulation of the cell bodies of NM neurons expressing soma-localized excitatory opsins was used to control NM release, while measuring NM indicator activity in the projection targets with fiber photometry. We evaluated indicators in at least two projection targets per NM with different axon innervation densities (e.g. the nucleus accumbens and basal amygdala for DA indicators), because the performance of indicators depends on affinities relative to NM concentration. To enable calibrated photostimulation we co-expressed soma-localized jGCaMP8s to monitor activity of NM neurons in response to photostimulation. This allowed us to calibrate photostimulation strength to physiologically-relevant activity levels. Epitope tags were attached to each indicator to quantitatively assess expression level using immunostaining. We assessed expression and surgical targeting with post-hoc slice-based histology and clearing-based whole-brain light-sheet microscopy, systematically registered to the Allen Institute's Common Coordinate Framework. We will share results on green dopamine sensors and discuss strategies for disseminating data and benchmarking protocols. We aim to provide a reproducible, standardized, and trusted resource to guide design of in vivo optical measurements of NMs.

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Poster

PSTR096: Probe Design and Engineering I

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the Peking-Tsinghua Center for Life Sciences and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

Title: Green and red GRAB sensors for monitoring endocannabinoid dynamics

Authors: *W. TEO[1], R. CAI[2], S. CAI[1], A. DONG[3], L. WANG[1], Y. LI[1];
[2]Sch. of Life Sci., [1]Peking Univ., Beijing, China; [3]Ctr. for Life Sci., Peking Univesity, Beijing, China

Abstract: Endocannabinoids (eCBs), including Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are key retrograde neuromodulators involved in diverse physiological processes. However, their distinct in vivo dynamics remain poorly understood due to the lack of tools with sufficient spatiotemporal resolution and molecular specificity. We previously developed a non-selective green GRAB sensor, eCB2.0, which detects both AEA and 2-AG with robust fluorescence changes (~300% $\Delta F/F_0$), enabling visualization of overall eCB dynamics in living animals. To distinguish between these two major eCBs, we now report the development of two selective green sensors: gAEA2.0, specific for AEA, and g2-AG2.0, selective for 2-AG. When expressing in vivo, we monitored both AEA and 2-AG signals during foot shock in the NAc core and BLA, whereas sucrose reward evoked time-locked 2-AG—but not AEA—signals in the VTA. We further investigated their dynamics in the NAc shell during administration of different psychoactive drugs, including nicotine and Δ9-tetrahydrocannabinol (Δ9-THC). Remarkably, nicotine evoked endogenous AEA signals, whereas Δ9-THC elicited only 2-AG responses. To enable simultaneous monitoring of AEA and 2-AG in behaving animals, we further engineered red-shifted versions, $rGRAB_{AEA}$ and $rGRAB_{2\text{-}AG}$, compatible with multiplexed imaging alongside their green counterparts. In summary, these selective and spectually-distinct eCB sensors provide powerful tools for real-time, dual-color monitoring of AEA and 2-AG in vivo, and open new avenues to dissect their individual contributions to brain function and behavior.

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Poster

PSTR096: Probe Design and Engineering I

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Title: Seeing NE in a New Light: Red and Far-Red GRAB Sensors for In Vivo Multiplex Imaging

Authors: *P. YANG[1], Y. CUI[1], Y. ZHENG[1], Y. HE[1], J. FENG[2], Y. LI[1];
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Abstract: Norepinephrine (NE) is a crucial neuromodulator involved in regulating arousal, stress, mood, and cardiovascular function. While green fluorescent $GRAB_{NE}$ sensors have enabled high-resolution visualization of NE dynamics *in vivo*, dissecting the complex interplay between NE and other signaling molecules requires spectrally distinct sensors—particularly red-shifted ones for multiplexed imaging. Red-shifted sensors further offer deeper tissue penetration, reduced phototoxicity and lower background autofluorescence for *in vivo* research. To meet this demand, we developed two red-shifted NE sensors using GPCR activation-based (GRAB) strategy: rNE2.0, a genetically encoded red fluorescent sensor, and HaloNE1.0, a chemigenetic far-red sensor using cpHaloTag and bright dye-ligand conjugate, together covering the emission spectrum from red to near-infrared. Both sensors showed good fluorescent responses to NE ($\Delta F/F_0$ >400%), nanomolar-range affinity, efficient membrane trafficking, and excellent molecular specificity with negligible cross-reactivity to dopamine in cultures. When expressing *in vivo*, both rNE2.0 and HaloNE1.0 reliably detects endogenous NE release in the lateral hypothalamus during tail suspension, with significantly improved signal-to-noise ratio over the previous published nLightR sensor. Moreover, the red-shifted sensors were optimized to eliminate blue-light-induced photoswitching, enabling compatibility with optogenetic tools and GFP-based indicators. Using HaloNE1.0, we successfully captured optogenetically evoked NE release in the medial prefrontal cortex (mPFC) of freely moving mice. To dissect the complex neuromodulation across sleep-wake cycles, combined with green and red GRAB sensors, we successfully monitored different dynamics for NE, serotonin, and acetylcholine simultaneously. Together, rNE2.0 and HaloNE1.0 expand the GRAB sensor palette for monitoring NE *in vivo*, and provide powerful tools for investigating neuromodulation in both physiological and pathological contexts.

Disclosures: P. Yang: None. **Y. Cui:** None. **Y. Zheng:** None. **Y. He:** None. **J. Feng:** None. **Y. Li:** None.

Poster

PSTR096: Probe Design and Engineering I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.09/NN6

Topic: J.04. Physiological Methods

Support: the National Natural Science Foundation of China 31925017
the National Natural Science Foundation of China 32525003
the New Cornerstone Science Foundation through the New Cornerstone Investigator Program
the Peking-Tsinghua Center for Life Sciences and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

the NIH BRAIN Initiative 1U01NS120824
Beijing Municipal Science & Technology Commission Z220009

Title: Development of chemigenetic fluorescence lifetime-based biosensors for quantitative detection of neurotransmitters

Authors: *Z. WANG[1], H. R. FARRANTS[2], E. R. SCHREITER[2], Y. LI[1];
[1]Peking Univ., Beijing, China; [2]Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: The nervous system is one of the most complex systems in the human body, comprising billions of neurons that communicate mainly through various neurotransmitters. Our lab has previously developed green and red fluorescent sensors for neurotransmitters based on the GPCR Activation-Based (GRAB) strategy, enabling sensitive and specific detection of neurotransmitter dynamics with high spatiotemporal resolution. However, these intensity-based sensors are inherently limited in their ability to quantify neurotransmitter levels due to intensity-related artifacts. Fluorescence lifetime, an intrinsic property of a fluorophore, is independent of fluorescence intensity and can be precisely measured, thus offering a promising approach for quantitative detection while maintaining high spatial and temporal resolution. In this study, we developed a series of new chemigenetic lifetime-based sensors, GRAB-WHalo sensors, using circularly permutated WHaloTag (HaloTag with a tryptophan mutation) for detecting various neurotransmitters. Specifically, the GRAB-WHalo-NE-0.4 sensor exhibits high response and affinity to norepinephrine when labeled with red fluorescent dye JF552-HTL and far-red fluorescent dye JF669-HTL. By transplanting the cpWHaloTag from GRAB-WHalo-NE-0.4 to other GPCRs, we extended this strategy to develop sensors for additional neurotransmitters, including dopamine, acetylcholine, and serotonin. These new sensors can be used with traditional green and red fluorescent sensors for multiplex imaging, and provide the possibility for quantitative detection of neurotransmitters.

Disclosures: Z. Wang: None. **H.R. Farrants:** None. **E.R. Schreiter:** None. **Y. Li:** None.

Poster

PSTR096: Probe Design and Engineering I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.10/NN7

Topic: J.04. Physiological Methods

Support: the New Cornerstone Science Foundation through the New Cornerstone Investigator Program
the National Natural Science Foundation of China (32525003 and 31925017)

Title: Development and applications of a tool kit of GRAB sensors for steroid hormones

Authors: *Y. YANG[1,2,3], J. GUAN[2,3], T. ZHAO[1,2,3], Y. LI[1,2,3];
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Abstract: Steroid hormones—a diverse class of cholesterol-derived signaling molecules—orchestrate critical physiological and pathological processes, including reproduction, development, metabolism, and behavior. This group includes sex hormones (e.g., **progesterone**, **estradiol**), corticosteroids (e.g., **corticosterone**), and **bile acids**. Despite their biological significance, investigating the spatial and temporal dynamics of steroid hormones *in vivo* remains a formidable challenge, largely due to the limited sensitivity, specificity, and spatiotemporal resolution of current detection techniques.
To address these limitations, we aim to generate a series of genetically encoded G-protein-coupled receptor activation-based (**GRAB**) sensors for steroid hormones. Starting with bile acids, we developed **BA0.5**, a sensor derived from the bile acid receptor TGR5. Leveraging structural and computational insights, we then re-engineered BA0.5's ligand-binding domain to create sensors for progesterone (**Prog0.6**), estradiol (**E2-0.6**), and corticosterone (**Cort0.5**). These sensors exhibited robust performance *in vitro*, with >600% $\Delta F/F_0$, nanomolar affinity, and good specificity. When expressed in the hypothalamus, these sensors dose-dependently responded to intraperitoneally administrated steroid hormones in ovariectomized (Prog0.6 and E2-0.6) and adrenalectomized mice (Cort0.5) via fiber photometry. More importantly, Prog0.6 detected endogenous hormone fluctuations in brains of intact females during innate behaviors, including sexual and maternal behaviors. Additionally, Cort0.5 revealed brain corticosterone dynamics under several stress conditions in intact mice. This work not only provides a powerful toolkit for real-time detection of steroid hormone signaling *in vivo*, but also establishes a generalizable strategy for developing GRAB sensors for a broad spectrum of steroid molecules.

Disclosures: Y. Yang: None. **J. Guan:** None. **T. Zhao:** None. **Y. Li:** None.

Poster

PSTR096: Probe Design and Engineering I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.11/NN8

Topic: J.04. Physiological Methods

Support: the NIH BRAIN Initiative (1U01NS113358)
the New Cornerstone Science Foundation through the New Cornerstone Investigator Program
the National Natural Science Foundation of China (32525003 and 31925017)

Title: Development and optimization of genetically encoded sensors for in vivo detection of oxytocin and arginine vasopressin

Authors: *L. GENG[1], H. WANG[1], B. LUO, Esq.[3], J. WAN[2], T. QIAN[4], Y. LI[1];
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Abstract: Oxytocin (OT) and Arginine vasopressin (AVP) are neuropeptides that play important roles in both the peripheral and the central nervous systems. Peripherally, OT regulates the contraction of uterine smooth muscle contraction and milk secretion, while AVP is essential for maintaining osmotic homeostasis. In the brain, OT is involved in pair-bonding and AVP contributes to stress-related behaviors. To understand their important functions, it is critical to be able to monitor OT and AVP dynamics in vivo with high selectivity, sensitivity and spatiotemporal resolution. To achieve this goal, we have developed genetically encoded green sensors for the detection of OT and AVP using the GRAB (G protein-coupled receptor activation–based) strategy, $GRABOT_{1.0}$ and $GRABAVP_{0.2}$. Through further optimization, we enhanced the sensors' response and signal-to-noise ratios (SNRs). Moreover, the new OT sensor shows higher selectivity to OT vs AVP. These sensors enable real-time detection of relevant neuropeptide release with fiber photometry recording upon hypertonic stimulation in freely moving mice. To facilitate multiplex imaging of different neuropeptides, we have also developed red fluorescence protein-based GRAB sensors. Together, these tools provide opportunity to a better understanding of neuropeptide functions in both physiological and pathophysiological conditions.

Disclosures: L. Geng: None. **H. Wang:** None. **B. Luo:** None. **J. Wan:** None. **T. Qian:** None. **Y. Li:** None.

Poster

PSTR096: Probe Design and Engineering I

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Program #/Poster #: PSTR096.12/NN9

Topic: J.04. Physiological Methods

Support: the National Natural Science Foundation of China (32525003 and 31925017)
the New Cornerstone Science Foundation through the New Cornerstone Investigator Program
the Peking-Tsinghua Center for Life Sciences and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences
the NIH BRAIN Initiative (1U01NS120824)

Title: In vivo multiplex imaging of dynamic neurochemical networks with designed far-red dopamine sensors

Authors: *Y. ZHENG[1], R. CAI[2], K. WANG[5], J. ZHANG[3], E. R. SCHREITER[6], Y. MU[5], Y. LI[4];
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Abstract: The human brain consists of billions of neurons that communicate primarily through neurotransmitters. Many brain functions depend on the coordinated regulation of multiple neurotransmitters and integrated control over downstream signaling pathways. To gain a better understanding of theses complex processes, there is an urgent need for tools that enable simultaneous monitoring of multiple neurotransmitters and neurochemicals in real time. Dopamine (DA) is one such crucial neurotransmitter, playing a pivotal role in regulating various physiological functions, including learning, motor control and reward. To address the challenge of multiplex imaging of DA with other neurochemicals, we developed a far-red DA sensor based on the G protein-coupled receptor activation (GRAB) strategy and a cpHaloTag-based chemigenetic approach, named $GRAB_{haloDA}$ (HaloDA1.0 in short). This sensor offers robust sensitivity, high specificity, rapid sub-second response kinetics, and an extended spectral range. The HaloDA1.0 is compatible with existing green and red sensors, including Ca^{2+} indicators, cAMP sensors, and optogenetic tools, making it highly versatile for multiplex imaging applications. It has been validated in cultured neurons, acute brain slices, and in behaving animals like zebrafish and mice. Furthermore, its modular and tunable design can be extended to other GPCRs and protein tags, providing a versatile platform for tracking multiple neurotransmitters and advancing our understanding of neurochemical regulation in health and disease.

Disclosures: Y. Zheng: None. **R. Cai:** None. **K. Wang:** None. **J. zhang:** None. **E.R. Schreiter:** None. **Y. Mu:** None. **Y. Li:** None.

Poster

PSTR096: Probe Design and Engineering I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.13/NN10

Topic: J.06. Computation, Modeling, and Simulation

Title: An optical platform for multiplexed fluorescence lifetime measurement of neural signals

Authors: *P. MA, A. BOWMAN;
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Abstract: Electro-optic fluorescence lifetime imaging microscopy (EO-FLIM) achieves wide-field fluorescence lifetime imaging with high frame acquisition rates using all-optical gating. It has enabled photon-efficient lifetime imaging of genetically encoded fluorescent indicators. When imaging FRET-opsin voltage indicators, for example, EO-FLIM has provided enhanced

detection of action potentials, rejection of motion artifacts, and resolution of subthreshold membrane potentials. However, studying neuronal computation requires simultaneous recording of multiple neuronal inputs and their post-synaptic effects on membrane potential to resolve intracellular computations and circuit dynamics. Here we aim to build a platform for EO-FLIM measurement of multi-component neuronal signals by lifetime and spectral multiplexing and unmixing. EO-FLIM optically gates the fluorescence decay into intensity bins to resolve lifetimes. To inform optimal gating functions and numbers of time bins for unmixing different neuronal signal components, we developed a Fisher information framework and used the Cramér-Rao lower bound to maximize separability of fluorescence lifetime decays. This framework showed that two optimal gating functions (with four optimally selected time bins) were sufficient to unmix two lifetime components. We verified that our system achieves theoretical limits from information analysis using simulation and maximum likelihood estimation (MLE). We are implementing these approaches into our latest EO-FLIM system that enables fast multi-phase acquisition as well as simultaneous calibration to time-correlated single photon counting (TCSPC) data. These methods will enable highly multiplexed measurements and analyses of neural signals in cells and circuits, empowering us to study higher-order rules of neural signaling.

Disclosures: P. Ma: None. **A. Bowman:** None.

Poster

PSTR096: Probe Design and Engineering I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR096.14/NN11

Topic: J.03. Anatomical Methods

Support: China Brain Initiative Grant 2022ZD0206700

Title: Robust and ultrabright chemical labeling enables rapid neural connectivity profiling in large tissue samples

Authors: *S. ZHONG[1], X. ZHANG[1], R. GONG[2], J. REN[3], M. LUO[2], R. LIN[1];
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Abstract: Mapping the neuronal connections between different brain areas is a major theme of neuroscience research. Given that individual neurons in a specific brain area often manifest unique morphologies, genetic heterogeneities and distinct functions, recent efforts have been directed at more precisely studying the complete morphologies of individual neurons at the whole-brain level in mammalian model organisms. Here, we present LINCS (*L*abel *I*ndividual *N*eurons with *C*hemical dyes and with controllable *S*parseness), a robust chemical labeling system that combines *in vivo* biotinylation produced by an engineered, solubility-enhanced biotin

ligase with rapid whole-mount staining. LINCS enables ultrabright, photostable, and cell-type-specific labeling of diverse tissue samples, ranging from entire mouse brains to whole adult mouse bodies. By integrating LINCS with tissue clearing and commercially available light-sheet microscopy, we established an efficient pipeline for rapid profiling of neuron projections in both the central and peripheral nervous systems. We further developed an adeno-associated virus system that achieves tunable and highly stable sparse labeling utilizing *in vivo* Cas9-mediated Cre knockout. Implementation of sparse labeling strategies empowers the LINCS-based pipeline for precise morphological reconstruction of individual neurons across the mouse brain. LINCS and LINCS-based single neuron reconstruction pipeline should accelerate single-neuron connectivity mapping and broaden the application of single-neuron reconstruction technologies in neuroscience research across mammalian models.

Disclosures: S. Zhong: None. **X. Zhang:** None. **R. Gong:** None. **J. Ren:** None. **M. Luo:** None. **R. Lin:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.01/NN12

Topic: J.06. Computation, Modeling, and Simulation

Title: Machine learning classification of brain dynamics using higher order network analysis: applications to mental health and neurodegeneration

Authors: *A. H. ASSADI;
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Abstract: Brain dynamics detection and classification from neurophysiological data is increasingly important for mental health, neurodegenerative diseases, and brain-computer interfaces. EEG provides non-invasive monitoring of brain activity, allowing investigation of dynamic states (e.g., emotions) and development of Brain Connectivity Dynamics (BCD) computational models. We developed novel Higher Order Network Analysis (HONA) algorithms for modeling BCD nonlinearity. HONA was implemented using publicly available EEG datasets in two domains: Attention-Deficit/Hyperactivity Disorder (ADHD) and Alzheimer's Disease (AD). For ADHD versus controls, HONA tested hypotheses on neural processing differences across emotions (positive, negative, neutral) for BCD classification (EEG: 62 channels×1536 samples×60 epochs per emotion). For AD versus controls, HONA studied diagnosis and patient-based BCD diversity under eyes-closed versus eyes-open conditions compared to healthy subjects (160 AD patients, 24 healthy controls). Implementation applied multiple Machine Learning methods to optimize BCD classification. Advanced statistical analyses using t-tests and ANOVA uncovered neurobiologically relevant EEG features, making HONA powerful for detecting brain dynamics including emotional states in ADHD and functional connectivity in AD. HONA achieved superior ADHD classification performance

versus existing methods ($p<0.001$, t-test). For AD, HONA revealed significant BCD differences between eyes-closed and eyes-open conditions ($p<0.05$, ANOVA). This work demonstrates HONA's critical role in feature selection for improving BCD accuracy in mental health studies, brain-inspired intelligent systems, and brain-computer interface technologies. All datasets comply with SfN ethical standards, methods are fully reproducible, and code is available on GitHub. HONA's mathematical foundations enable generalization to other neural data beyond EEG, offering promising advancement for brain functional connectivity applications in mental health and brain-like intelligent system design.

Disclosures: A.H. Assadi: None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.02/OO1

Topic: J.06. Computation, Modeling, and Simulation

Title: Order in disorder: regional brain turbulence aligns with cortical organization

Authors: *Y. SONG[1], A. IRAJI[2,3], J. CHEN[2,3];
[1]TReNDS, Atlanta, GA; [2]Georgia State Univ., Atlanta, GA; [3]Tri-institutional Ctr. for Translational Res. in Neuroimaging & Data Sci. (TReNDS), Atlanta, GA

Abstract: The problem of turbulence remains one of the most compelling challenges in modern physics, which can be understood as a highly efficient form of information processing and transfer. Recent work has revealed brain-wide, turbulence-like activity that appears to reflect the system's capacity for processing external stimuli (Deco & Kringelbach, 2020, Cell Rep.). However, because the brain is organized into modular and locally autonomous units (Bertolero et al., 2015, PNAS), regional information processing may exhibit unique aspects not addressed in previous studies. To investigate this, we analyzed resting-state fMRI data from fifty randomly selected subjects in the HCP S1200 release (MSMAll registration; ICA-FIX denoising; female = 24, mean age = 28.86, SD = 4.00). 360 cortical regions of interest (ROIs) were defined using the Glasser atlas, and for each ROI, we computed the local Kuramoto order parameter R, which quantifies how each vertex synchronizes with its neighbors. Amplitude turbulence (AT) was defined as the standard deviation of R across time and space, thereby capturing the temporal and spatial variability of local synchronization. High AT indicates frequent formation and dissolution of synchrony, while low AT reflects more stable synchronization. Across all ROIs, mean AT values were significantly greater than those obtained from phase-randomized surrogate data ($p <$ 1e-27, FWE-corrected), indicating that this turbulence does not merely result from random fluctuations. Spatial variability in AT across ROIs was also significant compared to the surrogate data ($p = 9.1e-51$), reflecting the locally specific nature of turbulent dynamics. Notably, the spatial distribution of mean regional AT (across all subjects) correlated with multi-task representation dimensionality ($r = 0.40$)—a measure of how richly task-specific features are

encoded at each ROI (Ito & Murray, 2022, Nature Neuro.)—and with cortical myelin content (r = 0.48). To characterize its global organization, we constructed a 360 × 50 matrix of regional AT values (rows = ROIs; columns = subjects) and applied principal component analysis. The first principal component closely matched the mean AT distribution, while the second component aligned strongly with the principal functional connectivity gradient, which reflects the unimodal-transmodal cortical hierarchy (r = 0.74). These results suggest that regional turbulence not only identifies areas of variable synchronization but also aligns with established dimensions of cortical organization. Moreover, it may reflect information processing tied to the heterogeneity and representational complexity of each region.

Disclosures: Y. Song: None. **A. Iraji:** None. **J. Chen:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

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Program #/Poster #: PSTR097.03/OO2

Topic: J.06. Computation, Modeling, and Simulation

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G0E0520N; G0C1920N
H2020 grant EBRAINS2.0

Title: Hyperalignment reveals shared information in monkeys' idiosyncratic fine-grained fMRI responses to a naturalistic movie

Authors: *Y. ZHANG[1], H. WANG[2], Q. ZHU[3], X. LI[2], J. HAN[1], G. JIAHUI[4], W. SHIM[5], R. KIM[5], M. GOBBINI[6], J. V. HAXBY[1], M. FEILONG[1], W. VANDUFFEL[2];
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Abstract: Hyperalignment is a functional alignment technique that maps individual brain activity patterns into a common, high-dimensional representational space, enabling more precise cross-subject comparisons than traditional anatomical alignment. Prior work in humans has demonstrated that hyperalignment enhances inter-subject decoding accuracy, uncovers shared representational structures across individuals, and improves the generalizability of neural models, especially using naturalistic paradigms such as movie viewing. However, comparable methodologies are underdeveloped in non-human primate research. Non-human primate brains serve as critical models for human brains, yet, as in humans, analyses across individuals are limited by anatomical and functional variability. In this study, we applied hyperalignment to

fMRI data from 12 rhesus monkeys while they watched the movie Monkey Kingdom. For each monkey, we derived a hyperalignment template using brain responses from the remaining 11 monkeys via a leave-one-out cross-validation. Individualized transformation matrices were computed using ridge regression on the first half of the movie and applied to the second half for validation analysis. Hyperalignment significantly improved inter-subject correlation and movie response prediction accuracy, particularly in the occipital, temporal, and lateral prefrontal cortices (Figure). Moreover, between-subject classification of hyperaligned brain responses achieved nearly 50% prediction accuracy in decoding which time point of the movie each monkey was watching. These results demonstrate that hyperalignment enables robust cross-monkey comparisons of fine-grained idiosyncratic neural representations and reveal high levels of specificity in population responses to naturalistic movie stimuli.

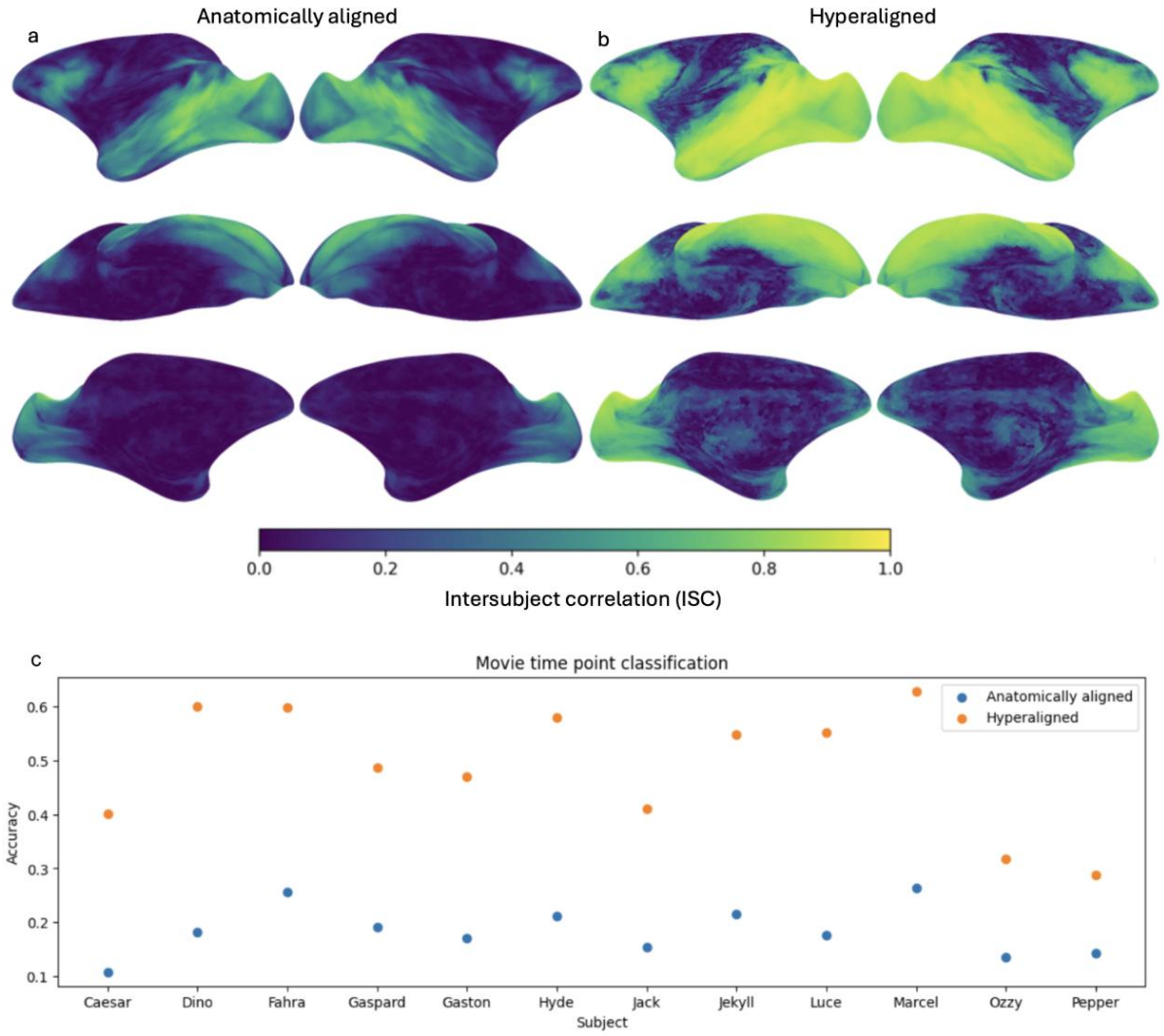


Figure. Hyperalignment increased mean vertex-wise intersubject correlations of movie-viewing response profiles from 0.25 (a) to 0.52 (b). (c) Movie time point classification accuracy comparison between anatomical alignment and hyperalignment for each subject.

Disclosures: Y. Zhang: None. **H. Wang:** None. **Q. Zhu:** None. **X. Li:** None. **J. Han:** None. **G. Jiahui:** None. **W. Shim:** None. **R. Kim:** None. **M. Gobbini:** None. **J.V. Haxby:** None. **M. Feilong:** None. **W. Vanduffel:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.04/OO3

Topic: J.06. Computation, Modeling, and Simulation

Support: NIMH Grant 5R01MH127199

Title: A functional template for the human brain

Authors: *M. FEILONG[1], Y. ZHANG[1], M. GOBBINI[2], J. V. HAXBY[1];
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Abstract: Different brains encode the same information as different topographic patterns. To study the information encoded in fine-grained patterns, such as representational geometry, it is necessary to hyperalign all brains to the same functional template, where these topographic idiosyncrasies are resolved. Traditionally, a separate functional template is created for each study. As a result, data from different studies are hyperaligned to different templates, making it difficult to compare the results across studies or to perform meta-analysis.
In this study, we created a standard functional template based on fMRI data collected from 300 participants during movie-watching, aggregated across four datasets. We built the template based on connectivity information, allowing it to support connectivity hyperalignment and be used in fMRI studies independent of stimulus content or timing. In other words, new participants do not need to watch the same movies as the 300 participants during fMRI data collection. Instead, they can watch different movies, perform tasks, or simply lie down and rest. We optimized the template using gradient descent, with the objective that in each searchlight (15 mm radius), the connectivity profiles of the template reflect the central tendency of the connectivity geometry of the 300 participants.
We validated the template with two movie datasets: the Raiders dataset, which was one of the datasets used for template creation; and the Forrest dataset, which was independent of the data used for template creation. For each dataset, we split the movie data into two halves for training (deriving hyperalignment transformations) and test (inter-subject correlation analysis), respectively. For both datasets, hyperalignment to the template greatly improved the quality of alignment across individuals, which is demonstrated by improved inter-subject correlation of brain response time series to the movie (Fig. 1).

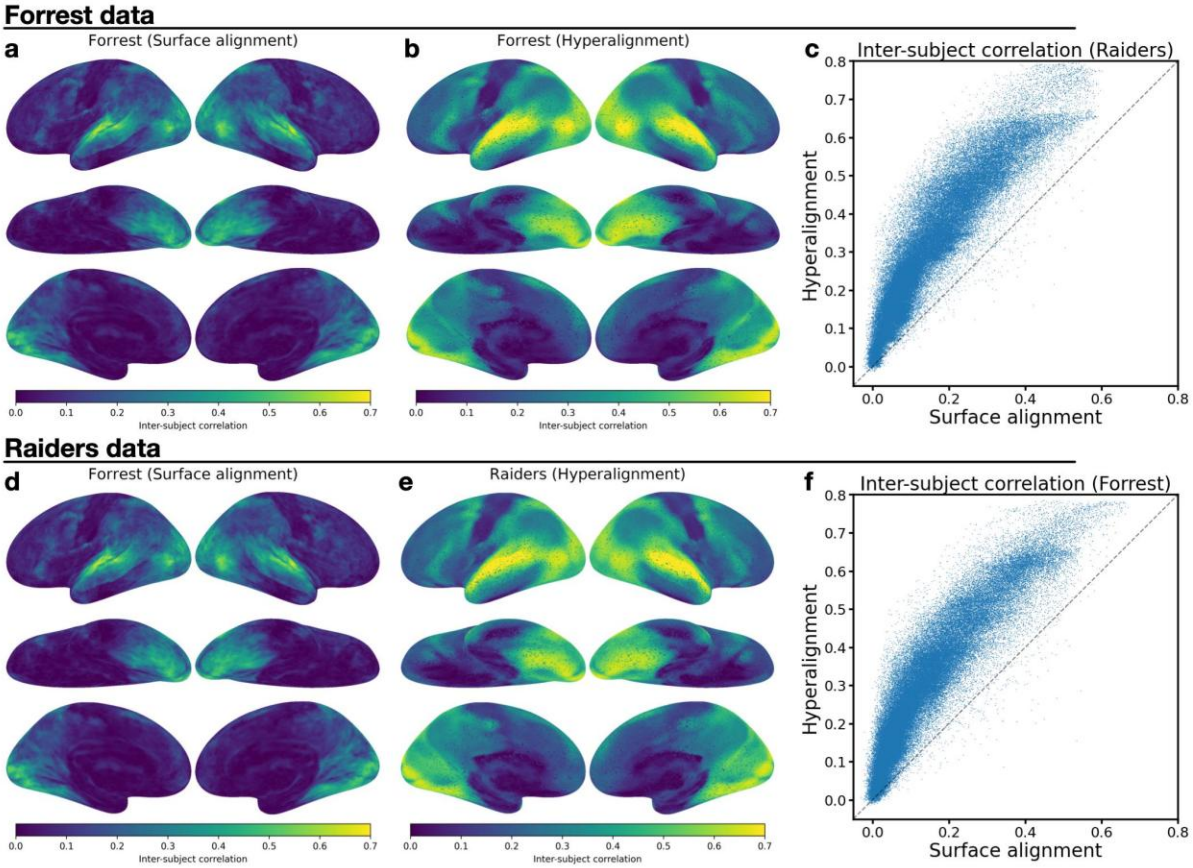


Fig. 1. Inter-subject correlation before and after hyperalignment to the template. For both the Forrest dataset (a–c) and the Raiders dataset (d–f), we observed significant improvements in inter-subject correlation after hyperalignment to the template (c and f).

Disclosures: M. Feilong: None. **Y. Zhang:** None. **M. Gobbini:** None. **J.V. Haxby:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.05/OO4

Topic: J.06. Computation, Modeling, and Simulation

Support: R01MH130899

Title: Precision functional mapping of individual human brain using resting-state and task-evoked fMRI data

Authors: *D. ZHI[1,2], C. NETTEKOVEN[3], L. SHAHSHAHANI[5], A. L. PINHO[3], J. DIEDRICHSEN[4], T. GE[1,2];

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Abstract: Over the past decade, advancements in precision functional mapping (PFM) of the human brain have enriched our understanding of inter-individual functional variability by delineating precise and reliable individual-specific brain parcellations. Unlike traditional group-based approaches, PFM captures each person's unique functional architecture, enabling the identification of subject-specific networks. This holds significant clinical promise for personalized diagnosis and treatment of neurological and psychiatric disorders. However, most existing PFM methods derive individual parcellations solely from resting-state fMRI data (Kong et al., 2019, Gordon et al., 2017), while task-evoked data are typically reserved for evaluation. The potential of task fMRI to contribute directly to individualized parcellation remains underexplored. Here, we hypothesize that resting-state and task-evoked data contain complementary information about brain functional organization, and that integrating both will yield more functionally meaningful individual parcellations than resting-state data alone. To test this, we introduce a new PFM method that fuses rest and task fMRI data to generate individual parcellations. The model is built upon our previous hierarchical Bayesian parcellation (HBP) framework (Zhi et al., 2025), integrated with a novel spatial architecture based on a multinomial restricted Boltzmann machine (m-RBM). Specifically, this model (1) optimally integrates different data sources at the individual level and (2) effectively captures local spatial dependencies of cortical regions, enabling reliable parcellation even with limited data. We then test our hypothesis on three fMRI datasets, HCP (Van Essen et al., 2013), MSC (Gordon et al., 2017), and a new collected deep-phenotyping dataset (Du et al., 2024), that having both rest and task data for the same subjects. For each dataset, we generated parcellations using rest-only and fused (rest and task) data of equal duration. To ensure a fair comparison, all parcellations were generated from the same group map and the number of parcels was fixed to 15. We then assessed the performance of the parcellations using DCBC (Zhi et al., 2022), task inhomogeneity, and mean task activation z-values. The Dice coefficient was used to assess the network correspondence between the rest-only and fused parcellations. Across all datasets, we found that the fused parcellations show consistent advantage in functional localization compared to rest-only parcellations, offering a promising alternative perspective on precision functional mapping at the individual level.

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Poster

PSTR097: Computational Approaches in fMRI or EEG I

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.06/OO5

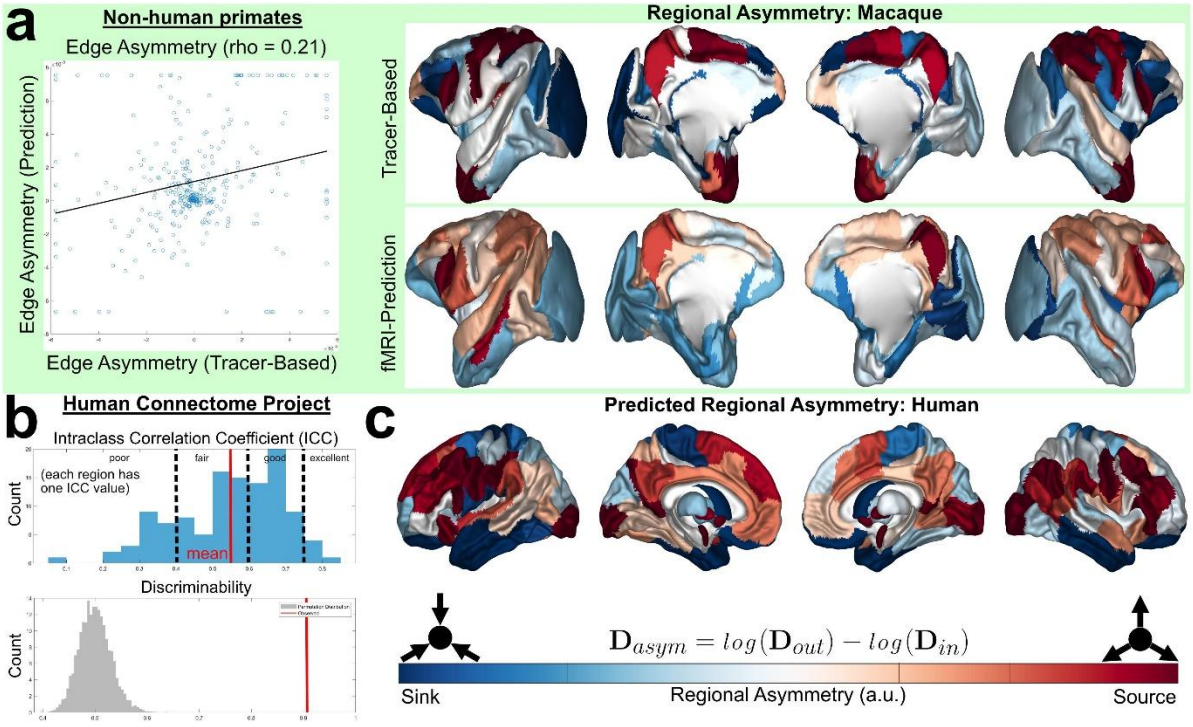
Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant R01EB022717
NIH Grant R01AG0621
NIH Grant R01AG072753
NIH Grant R56AG082087
NIH Grant RF1AG062196

Title: Inferring directed structural connectivity with a structure-function model in humans and non-human primates

Authors: *B. S. SIPES, A. RAJ;
Radiology, Univ. of California, San Francisco, San Francisco, CA

Abstract: Diffusion MRI (dMRI) tractography is used to estimate structural connectivity (SC) between brain regions *in vivo*, but it lacks directional information about white matter pathways. Here, we introduce a computational framework to infer directional SC by integrating dMRI-derived SC with fMRI. Using a Higher-Order Network Diffusion (HONeD) model [1], we introduce asymmetry to the SC matrix (C) via a similarity transform (ACA^{-1}), where A is a learnable diagonal matrix that encodes region-specific asymmetry. Our model has one asymmetry parameter for each region, and two global parameters determining the amount of signal diffusion through the network. We then fit the HONeD model parameters (via hybrid gradient descent and genetic optimization) to predict the empirical functional covariance in fMRI time series. We validate our approach in 9 macaques (8 *Macaca mulatta*, 1 *Macaca fascicularis*) using a symmetric dMRI-derived SC and fMRI [2], finding a significant correspondence between predicted asymmetry and tracer-based directionality from CoCoMac [3] (Spearman's rho = 0.21, $p < 10e\text{-}4$; Fig 1a). We then evaluated our method's test-retest reliability using 31 subjects from the Human Connectome Project (HCP) [4], and we observed fair to excellent reliability across regions (ICC = 0.55 +/- 0.15) and high subject discriminability (Discriminability = 0.91, $p = 0$, permutation test; Fig 1b). Finally, we evaluated inferred SC directionality across 770 HCP subjects. The strongest network sinks included the caudate and limbic areas; conversely, the strongest network sources included the amygdala, nucleus accumbens, visual, motor, and default mode areas (Fig 1c). This framework offers a promising method to infer directional white matter pathways, with applications in cognitive neuroscience and neurodegenerative diseases involving directed protein spread, such as Alzheimer's disease.
References: [1. Sipes et al., 2025. IEEE (ISBI).] [2. Shen et al., 2019. OpenNeuro.] [3. Stephan et al., 2001. Philos. Trans. R. Soc. A.] [4. Van Essen et al., 2013. Neuroimage.]



Disclosures: B.S. Sipes: None. **A. Raj:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.07/OO6

Topic: J.06. Computation, Modeling, and Simulation

Support: State Key Program of National Natural Science of China (grant number 82130118

Title: Decoupling MCI-Specific Brain and Cognitive Pathologies via Deep Learning

Authors: *B. PENG[1], Z. ZHANG[2];
[1]State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China;
[2]State Key Lab. of Cognitive Neurosci. and, Beijing, China

Abstract: Resolving the neuroanatomical ambiguity of mild cognitive impairment (MCI)—where age-related and other non-pathological factors, along with pathological neurodegeneration, exhibit overlapping manifestations—presents a critical unmet challenge due to the syndrome's clinical heterogeneity and the technical limitations of conventional mass-univariate neuroimaging analyses in handling latent confounders. Leveraging data from the Beijing Aging Brain Rejuvenation Initiative (BABRI, n = 918) and the Alzheimer's Disease

Neuroimaging Initiative (ADNI, n = 1293), this study employed a conditional variational autoencoder (CVAE) to achieve three key objectives: (1) systematically distinguish between aging-related, shared cognitive decline and MCI-specific cognitive impairments; (2) implicitly disentangle latent, unknown confounding effects to identify MCI-specific structural brain alterations; and (3) construct individualized scores for predicting the risk of conversion to Alzheimer's disease (AD). The results showed that CVAE could effectively extract MCI-specific features, revealing episodic memory, attention, and executive function as the core domains of disease-specific impairments, and identified MCI-specific brain regions including the middle temporal lobe, medial temporal lobe, frontal lobe, and limbic system, as well as the cerebellum. The robustness of this cross-cohort structural-cognitive impairment association model across cohorts was validated in the ADNI dataset. In addition, the predictive model based on CVAE-derived disease-specific brain structures demonstrated superior performance (AUC=0.74) in estimating longitudinal risk of AD conversion, compared to models based on whole-brain structures (AUC=0.67) and classical biomarkers (AUC=0.70). This computational neuroscience framework establishes a new paradigm for disentangling pathogenic neurodegeneration from physiological aging processes.

Disclosures: B. Peng: None. **Z. Zhang:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.08/OO7

Topic: J.06. Computation, Modeling, and Simulation

Support: Korea NRF MSIT RS-2024-00335670

Title: Connectome Atlas Transformation (CAT) model: Addressing brain atlas inconsistency in functional connectomes via a transformation framework

Authors: *Y. KIM[1], E. YANG[1], K. R. YOO[2,3,4];
[1]Samsung Advanced Inst. for Hlth. Sci. and Technol., [2]Digital Hlth., Sungkyunkwan Univ., Seoul, Korea, Republic of; [3]AI Res. Center, Data Sci. Res. Institute, Res. Inst. for Future Med., Samsung Med. Ctr., Seoul, Korea, Republic of; [4]Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci. (IBS), Suwon, Korea, Republic of

Abstract: Human connectomes derived from non-invasive MRI model brain architecture at the macroscopic scale as graph-like structures, where nodes represent distinct brain regions and edges reflect their connections. These regions are defined by various parcellation methods, producing atlases that vary in number, size, and topology. While connectome-based biomarkers have emerged as useful tools to capture individual differences and neuropsychiatric conditions, atlas-dependent variability poses challenges for reproducibility and generalization. Although large-scale datasets such as Human Connectome Project and UK Biobank now provide

preprocessed connectomes, the lack of consensus on atlas selection limits comparing results from different studies. To overcome this limitation**,** we propose a connectome atlas transformation (CAT) model that maps connectomes obtained with different brain atlases, building on a prior framework (Yoo et al., 2022)**.** CAT consists of three steps: (1) extracting components from source representations, (2) transforming them into components of target representations, and (3) reconstructing whole-brain connectomes from transformed target components. We constructed and evaluated CAT using data from 1,102 individuals from the Autism Brain Imaging Data Exchange dataset in Preprocessed Connectome Project, starting with healthy (n = 568) and ASD cohorts (n = 534) to assess clinical applicability. This study included AAL and Harvard-Oxford (anatomical), Dosenbach and Craddock 200/400 (functional), and Eickhoff-Zilles (cytoarchitectonic) atlases. Model performance was assessed with similarity (r), *MSE*, and q^2 between empirical and transformed target connectomes in LOOCV. CAT successfully transformed connectomes across atlases, demonstrating its reliability for cross-atlas mapping. When anatomical and cytoarchitectonic atlases were targets, the model accurately transformed them from all source atlases ($r \approx 0.70$-0.72; MSE ≈ 0.017-0.018; $q^2 \approx 0.16$-0.18). In contrast, targeting the functional Dosenbach led to less accurate transformations ($r \approx 0.51$-0.53; MSE ≈ 0.010; $q^2 \approx 0.09$) despite its effectiveness as a source ($r \approx 0.70$-0.71; MSE ≈ 0.016-0.018; $q^2 \approx 0.13$-0.15 when predicting anatomical and cytoarchitectonic). Craddock200/400 showed moderately successful mapping in both directions ($r \approx 0.61$-0.68; MSE ≈ 0.010-0.018; $q^2 \approx 0.11$-0.15). These findings highlight that atlas type and direction may influence transformation outcomes. Overall, CAT performs reliably across various brain atlases, offering a practical approach to improve data harmonization and reproducibility in multi-atlas neuroimaging studies.

Disclosures: Y. Kim: None. **E. Yang:** None. **K.R. Yoo:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.09/OO8

Topic: J.06. Computation, Modeling, and Simulation

Support: JSPS KAKENHI JP24K15188
JSPS KAKENHI JP25H01365
MIC grant JPMI00316

Title: Whole-brain fMRI dynamics of sudden visual insight highlight the superior colliculus

Authors: T. MURATA[1], K. KUNISHIGE[2], S. SENO[2], I. OHZAWA[2], K. KANEKO[3], T. YANAGIDA[1], M. HARUNO[1], ***K. HOSODA**[1];
[1]Natl. Inst. of Information and Communications Technol., Osaka, Japan; [2]Osaka Univ., Osaka, Japan; [3]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Sudden insight, often associated with “Eureka” experiences, has fascinated neuroscientists for decades. One compelling experimental paradigm to study sudden visual insight is Mooney image recognition, which involves highly degraded black-and-white stimuli that initially appear meaningless but can abruptly be perceived as coherent objects, including non-face images. While previous studies have highlighted cortical involvement (e.g., unconscious top-down influences from parietal and frontal regions) by comparing recognized and unrecognized states via functional magnetic resonance imaging (fMRI) or electroencephalography (EEG), the whole-brain dynamics in the transition from non-recognition to recognition remain poorly understood, particularly the contributions of subcortical and brainstem structures. Here, we demonstrate that this transition can be effectively characterized by three large-scale functional activation patterns, and that the superior colliculus (SC) emerges as a key structure with a distinct temporal activation profile during insight. We analyzed whole-brain fMRI data consisting of 41,446 timepoints collected from 14 participants engaged in a Mooney image recognition task. The data were represented by time series from 148 brain subregions using a pre-defined functional parcellation. Functional clustering revealed that these time series grouped into three robust clusters: stimulus-driven activation, suppression, and recognition-associated patterns. The third cluster, associated with recognition, encompassed brainstem and midbrain regions, with the SC showing a sharp activation peak just prior to recognition response. Empirical dynamic modeling further revealed that the SC was the only region among the 148 subregions that exhibited mutually positive directed interactions with all three functional clusters. Higher-resolution analysis indicated that SC was not directly involved in sensory input or motor output, but was functionally embedded within higher-order cortical interactions mediating sensorimotor transformation. Our findings provide compelling experimental evidence that the SC plays a critical role in coordinating whole-brain dynamics underlying sudden insight and prompt a reevaluation of this evolutionarily conserved structure as an unconscious yet key player supporting high-level cognitive processing. Moreover, this study presents an analysis approach for examining dynamic interactions involving non-cortical regions using noninvasive fMRI, building upon the rich foundation of cortex-centered research to broaden the scope of human neuroscience.

Disclosures: T. Murata: None. **K. Kunishige:** None. **S. Seno:** None. **I. Ohzawa:** None. **K. Kaneko:** None. **T. Yanagida:** None. **M. Haruno:** None. **K. Hosoda:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.10/OO9

Topic: J.06. Computation, Modeling, and Simulation

Support: Cognitive Neuroimaging Centre D821
Human Potential Programme Prenatal/Early Childhood Grant H22P0M0002

Title: Uncovering the neural basis of inner speech and emotional connectedness through multimodal EEG-fMRI analysis

Authors: *B. RAGHAVAN;
Nanyang Technological Univ., SIngapore, Singapore

Abstract: The Cognitive Neuroimaging Centre supports advanced research using simultaneous EEG and fMRI to examine complex brain functions with high spatial and temporal resolution. This abstract highlights research that has leveraged these multimodal capabilities to explore both language processing and interpersonal emotional dynamics.
We examined covert speech, the internal generation of words without vocalisation, which plays a critical role in cognitive functioning and is highly relevant to brain-computer interface applications. Healthy male participants performed overt and covert word-repetition tasks during EEG-fMRI acquisition. Spatial-temporal features derived from fMRI-informed EEG source localisation revealed significant activation in regions including the left precentral gyrus, supplementary motor area and left putamen. Earlier activation in the left putamen and weaker functional connectivity with speech-motor areas during covert speech suggested distinct neural processing mechanisms compared to overt speech.
We also explored emotional connectedness in genetically identical individuals. Pairs of monozygotic twins were exposed to familiar and unfamiliar audiovisual stimuli while undergoing either EEG or fMRI in a resting state, with roles later reversed. Neural responses in passive twins mirrored those of their active co-twins for familiar stimuli. Preliminary fMRI data showed increased activation in areas such as the posterior cingulate and occipital cortex, particularly in non-electromagnetically shielded settings. These results pointed to potential neurobiological markers of shared emotional experiences.
Together, these studies highlighted the versatility of EEG-fMRI integration in our multimodal neuroimaging approach to elucidate the neural mechanisms underlying both cognitive and emotional processes. We are currently conducting in-depth source-level analysis to explore functional and effective connectivity patterns and applying machine learning techniques to extract robust neural signatures. These efforts would deepen our understanding of spatiotemporal brain dynamics and pave the way for potential clinical applications in diagnosing and monitoring neuropsychiatric and communication disorders.

Disclosures: B. Raghavan: None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.11/OO10

Topic: J.06. Computation, Modeling, and Simulation

Support: Internal Department Funding

Title: Cholinergic Disruption Reshapes Neural Population Geometry During Memory Encoding

Authors: *Z. LEI[1], B. C. LEGA[2];
[1]Neurolog. Surgery, [2]Neurosurg., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Cholinergic signaling plays a critical role in memory and is profoundly disrupted in conditions such as Alzheimer's disease. Scopolamine, a muscarinic antagonist, models this disruption by impairing memory and altering neural oscillatory activity. However, interpreting scopolamine-induced neural oscillations is challenging due to non-drug-related confounding effects, especially at higher frequencies. To disentangle drug-specific neural signatures from recall-related activity, we applied demixed principal component analysis (dPCA) to intracranial EEG (iEEG) recordings from 14 epilepsy patients who completed both scopolamine and placebo sessions during free (n=9) or associative (n=5) recall tasks. We analyzed trial-averaged power across five frequency bands (slow theta, theta, alpha, beta, and gamma) from hippocampal and temporal electrodes, using bootstrapped, trial-balanced data to iteratively extract principal components (PCs) that reflect the effects of drug, behavior, time, and their interaction. The variance explained from PCs attributed to drug, recall, and their interaction is similar across bootstrapped dPCA, demonstrating robust cross-conditional modulation. Linear support vector machines (SVMs) trained on dPCA-decoded components reliably classified both drug and recall conditions, with accuracies exceeding 60% across frequency bands and surpassing 90% in lower frequencies. Pairwise Procrustes analysis revealed greater variability in the distribution of decoded data at lower frequencies, suggesting that neural representations in these bands are more susceptible to trial-by-trial fluctuations compared to those at higher frequencies. To characterize representational geometry, we projected bootstrapped data into drug- and recall-specific subspaces and evaluated each using the decision functions derived from trained SVM hyperplanes. By examining correlations between projections in drug and recall-specific decoding subspaces, we assessed how scopolamine and memory retrieval independently and jointly influence neural population coding. Aligning decoding subspaces across bootstrapped datasets revealed stable, low-dimensional axes associated with drug and recall conditions, indicating that these cognitive and pharmacological states are encoded along distinct and reproducible neural dimensions. These findings provide condition-specific representational formats even amid trial-level variability, supporting a quantitative framework for tracking how neuromodulatory interventions alter memory-related population structure.

Disclosures: Z. Lei: None. **B.C. Lega:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.12/OO11

Topic: J.06. Computation, Modeling, and Simulation

Support: NRF Grant RS-2024-00341889

Title: Non-invasive hippocampal stimulation via dual high-frequency electrode pairs for Parkinson's disease

Authors: *Y. SEO;
Korea Res. Inst. of Standards and Sci., Daejeon, Korea, Republic of

Abstract: Introduction Prominent brain stimulation technologies include transcranial magnetic stimulation, electrical stimulation, and ultrasound stimulation. However, these methods are not without their own side effects and limitations. As a result, there is a pressing need for the development of safer and more effective technologies capable of selectively targeting deep brain regions. Such advancements should aim to minimize side effects while surpassing the limitations of existing brain stimulation techniques. **Methods** To achieve non-invasive and effective stimulation of the desired deep brain regions, multiple electric fields with different high frequencies are applied. This study investigates whether it is possible to achieve effective stimulation of the desired deep brain regions by utilizing two or more pairs of electrodes. The target area for deep brain stimulation is the left hippocampus, which is associated with severe movement disorders such as Parkinson's disease. For non-invasive and effective stimulation, two pairs of electrodes are used, each applying different frequencies of 2000 Hz and 2005 Hz, respectively. The electric current amplitude is set at 1 mA. The electrode pairs are positioned as follows: one pair (E1) at FT9 and T8, and the other pair (E2) at TP9 and PO8, based on the 10-10 EEG system. **Results** The simulation results of non-invasive deep brain stimulation targeting the left hippocampus, associated with Parkinson’s disease, are shown in Figure 1. As illustrated in Figure 1, two electric fields are generated through the temporal interference of the yellow and blue electrodes, resulting in brain stimulation (gold color). Notably, the results confirm that the left hippocampus, the target deep brain region (red color), is being stimulated non-invasively and effectively. **Conclusions** This study demonstrates that it is not only possible to effectively stimulate the deep brain region, the hippocampus, using two pairs of electrode pads, but also that the method can be applied to other desired deep brain regions.

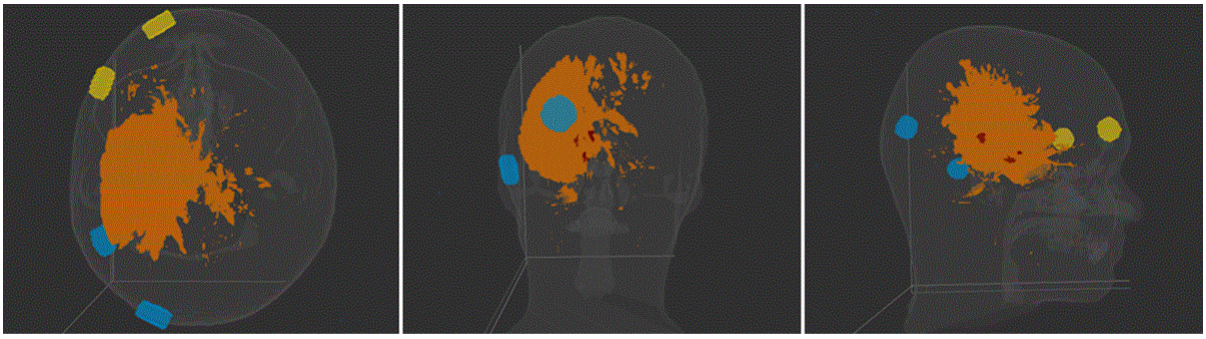


Figure 1. Simulation results of neurostimulation in the left hippocampus via two pairs of electrodes

Disclosures: Y. Seo: None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.13/OO12

Topic: J.06. Computation, Modeling, and Simulation

Support: Alfred P. Sloan Research Fellowship in Neuroscience FG-2022-19032

Title: Surprising narrative events elicit convergent responses in brain network dynamics, subjective reports, and large language models

Authors: *Z. ZHANG[1,2], J. PARK[2], I. SUMME[2], K. GOLLAPUDI[2], Y. LEONG[2], M. D. ROSENBERG[2];
[1]The Univ. of Chicago, Chicago, IL; [2]Dept. of Psychology, Univ. of Chicago, Chicago, IL

Abstract: Linguistic surprise occurs when incoming words violate expectations formed from prior context. For example, when we hear a story, we are surprised when unfolding events do not align with our expectations. Here we ask whether large language models (LLMs) represent event-level surprise similarly to humans. Two story stimuli—Paranoia (22 min; Finn et al., 2015) and How to draw (12 min; from The Moth)—were manually segmented into 61 and 36 events, respectively. For each word in the two stories, we quantified LLM surprise by prompting the model to predict upcoming words. We then operationalized LLM surprise as the dissimilarity between LLM's internal embeddings of the predicted and actual text. To measure human surprise, we collected continuous self-reported ratings while new participants (N=30) listened to the same stories. We used predictions of a model built from a recently developed measure of fMRI network dynamics to quantify neural surprise (Faskowitz et al., 2020). This model—the surprise edge-fluctuation-based predictive model—has been shown to predict surprise in both active learning and passive viewing tasks that differ from the story listening task in both context (e.g., non-linguistic stimuli) and measurement approach (e.g., reduced Bayesian model and categorical summary models quantifying surprise; Zhang et al., 2024). We reanalyzed openly available fMRI data collected from two separate groups of participants as they listened to Paranoia (N=20; Finn et al., 2015) and How to Draw (N=8; LeBel et al., 2023). Because we are interested in surprise to narrative events, we calculated event-level LLM, human, and neural surprise by averaging these measures within-event. LLM and human surprise were correlated across events in both stories. A regression model revealed that dynamics in the brain model of surprise EFPM were related to both behavioral ratings and LLM surprise in Paranoia ($t=2.150$, $p=0.038$; $t=2.689$, $p=0.026$) but not How to Draw ($t=-1.356$, $p=0.252$; $t=-0.500$, $p=0.673$), accounting for lower level features in the stories. Taken together, these results suggest that LLMs and humans predict the same events as surprising. Our findings highlight the value of brain-network-based models for identifying cognitive state dynamics across contexts and LLMs' potential in modeling human surprise to narrative events.

Disclosures: Z. Zhang: None. **J. Park:** None. **I. Summe:** None. **K. Gollapudi:** None. **Y. Leong:** None. **M.D. Rosenberg:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.14/OO13

Topic: J.06. Computation, Modeling, and Simulation

Support: National Science Foundation BCS-2043740 to M.D.R.
resources provided by the University of Chicago Research Computing Center

Title: Engagement strengthens functional attention networks and neural representations of naturalistic stimuli

Authors: *A. CORRIVEAU[1,2,3], J. KE[4], M. D. ROSENBERG[3,2];
[2]Inst. for Mind and Biol., [3]Dept. of Psychology, [1]Univ. of Chicago, Chicago, IL; [4]Yale Univ., New Haven, CT

Abstract: Subjective engagement to naturalistic content such as movies and podcasts involves the maintenance of attention over time. However, the extent to which functional brain connectivity and activity signatures of sustained attention are reflected in subjective engagement states is not fully understood. We hypothesized 1. that functional networks trained to predict objective measures of sustained attention task performance should also capture fluctuations in subjective engagement and 2. that moments of heightened engagement should increase fidelity of stimulus representations in the brain. To test these questions, we collected functional MRI data from 60 participants who performed visual- and auditory-relevant continuous performance tasks (CPTs) and were presented four naturalistic narratives: two audio-visual movies, one silent movie, and one podcast. Following scanning, participants reported continuous subjective engagement for all narrative stimuli using a slider bar. Functional MRI data were parcellated into 432 cortical and subcortical regions (ROIs). To test if dynamics of the same functional networks predicted objective sustained attention performance and subjective narrative engagement, we identified pairs of brain regions whose activation similarity, or co-fluctuations, differentially tracked successful performance vs. lapses during CPTs. Co-fluctuation dynamics in this network correlated with self-reported engagement in three of four narratives (ps<.01), suggesting that this network tracks naturalistic engagement. Next, we investigated the role of engagement in stimulus representational fidelity in the brain. Narratives were segmented to obtain meaningful representational units—visual narratives were parsed by scene and auditory narratives were parsed by sentence. Stimulus representations were defined as sentence embeddings for scene descriptions and sentence transcripts. Neural representations were defined as voxel-level beta estimates for all scenes and sentences using least squares separate modelling. Neural and stimulus representational Euclidean distance dissimilarity matrices (RDMs) were calculated for high- and low-engagement scenes. Results revealed that engagement increased the strength with which neural representations reflected stimulus features in large swaths of the brain during all narratives, including prefrontal and visual areas. These findings suggest that neural signatures of sustained attention, including co-fluctuation and representational strength in functional networks, capture engagement during naturalistic narrative viewing.

Disclosures: A. Corriveau: None. **J. Ke:** None. **M.D. Rosenberg:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.15/OO14

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH T32 Grant HD07475

Title: Fine-scale dynamics of age-related decline in functional network segregation

Authors: *S. A. CUTTS[1], R. PATEL[1], C. HUGHES[1], R. FRENCH[1], A. KRENDL[1], O. SPORNS[2];
[2]Psychological and Brain Sci., [1]Indiana Univ., Bloomington, IN

Abstract: Greater network segregation in functional connectivity (FC) has been linked to better cognitive performance. In contrast, older adults (OA) often exhibit reduced segregation, which has been proposed as a potential mechanism underlying age-related cognitive decline [1]. Network segregation is often studied using full recording sessions, but it remains unclear how temporal fluctuations and differences in scan length influence age comparisons. Previous work has shown that temporal variations in FC, measured by unwrapping FC into pairwise co-fluctuation time series [2], emerge as functional systems that appear transiently during a scan [3]. Here, we directly test whether moments expressing greater network segregation or functional system specificity relate to stronger age differences and cognitive function. We analyzed resting-state fMRI data from 91 younger adults (YA; mean age = 22.2) and 62 OA (mean age = 72.9) collected at Indiana University. Connectivity components (FCc) were created from moments matching system expressions and assessed for age differences in segregation [4]. We compared mean system segregation of full FC, examined the frequency of transient emergence of functional systems, and used cartographic profiles to assess time spent in segregated versus integrated states [5]. OA showed lower overall segregation in full FC than YA ($p<0.0001$), with differences localized to transient fluctuations. Across both age groups, segregation computed on FCc of specific functional systems was greater than full FC and varied across individuals. While system frequencies were similar, OA spent less time in globally segregated than integrated states ($p<0.0001$). Notably, OA showed localized, momentary increases in segregation via FCc analysis that revealed stronger brain-cognition associations than full FC. In particular, moments expressing a system involved in executive function, the frontoparietal network (FPN), were significantly correlated with a composite cognition score ($r=0.30$, $p=0.019$), which was absent using full-scan measures. These findings suggest that age-related decline in segregation can occur within transient brain dynamics. Our approach reveals that OA can achieve higher network organization than traditional measures suggest, and these segregated moments relate more strongly to cognitive function. This demonstrates how brain network dynamics can enable more uniform comparisons across age groups and uncover relationships unseen in full-session data.
[1] Wig, G. S. (2017) TiCS; [2] Faskowitz, J. (2020) Nat. Neurosci.; [3] Sporns, O. (2021) Netw Neurosci.; [4] Cutts, S.A. (2023) Cereb. Cortex; [5] Shine, J.M. (2016) Neuron

Disclosures: S.A. Cutts: None. **R. Patel:** None. **C. Hughes:** None. **R. French:** None. **A. Krendl:** None. **O. Sporns:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.16/OO15

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant F31AA032179
NIH Grant R01MH121095

Title: Moment-to-moment network dynamics align to support flexible brain state engagement and cognitive control transdiagnostically

Authors: *J. YE[1], S. MEHTA[1], M. KHAITOVA[1], J. ARORA[1], F. TOKOGLU[1], C. LACADIE[1], A. GREENE[2], R. T. CONSTABLE[1], D. SCHEINOST[1];
[1]Yale Univ., New Haven, CT; [2]Brigham and Women's Hosp., Boston, MA

Abstract: Neural variability, or variation in brain signals, facilitates dynamic brain responses to ongoing demands. Our previous work showed that variability in the engagement of recurring brain state (i.e., SEV) can predict cognitive control in previously unseen individuals. However, it remains unclear whether moment-to-moment flexibility in brain state engagement supports fluctuations in cognitive control over time. Here, we investigated this question using functional magnetic resonance imaging (fMRI) and out-of-scanner behavioral data from a transdiagnostic dataset (Yale dataset) recruiting healthy controls (HCs; N=92) and patients with mood, substance, or neurodevelopmental disorders (N=145). Using functional connectomes from a stop-signal task and 10-fold connectome-based predictive modeling, we identified three sets of brain networks predicting individual differences in SEV ($r=0.37$, $p=0.002$), cognitive flexibility (assessed by the shift subscale from the Behavioral Rating Inventory of Executive Function; $r=0.25$, $p<0.001$), and cognitive stability (estimated by Color Word response time in the incongruent condition; $r=0.27$, $p<0.001$) transdiagnostically. We tracked these networks' instantaneous engagement with edge timeseries to investigate how moment-to-moment SEV and cognitive control fluctuated over time. Correlation between continuous SEV and cognitive control network engagement assessed whether flexibility in brain state engagement supported variations in cognitive control. During rest, moments of more flexible brain state engagement aligned with networks dynamics associated with better cognitive flexibility ($t(236)=-14.95$, $p<0.001$) and cognitive stability ($t(236)=-25.12$, $p<0.001$) transdiagnostically. Further, we validated this relationship in two independent, transdiagnostic datasets (Consortium for Neuropsychiatric Phenomics dataset (CNP; N=225) and Transdiagnostic Connectome Project (TCP; N=163)) and obtained consistent findings ($ps<0.001$). Interestingly, group comparison revealed that the alignment between SEV and cognitive flexibility network dynamics was weaker in patients than in HCs (Yale dataset: $t(235)=2.76$, $p=0.006$; CNP: $t(223)=2.90$, $p=0.004$; TCP:

t(177)=1.97, p=0.0499). Altogether, these findings demonstrated that moment-to-moment flexibility in brain state engagement is relevant for supporting changes in cognitive control across three independent datasets. Importantly, we found that patients might experience a greater difficulty in flexibly recruiting recurring brain states to effectively respond to evolving demands in cognitive flexibility.

Disclosures: J. Ye: None. **S. Mehta:** None. **M. Khaitova:** None. **J. Arora:** None. **F. Tokoglu:** None. **C. Lacadie:** None. **A. Greene:** None. **R.T. Constable:** None. **D. Scheinost:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.17/OO16

Topic: J.06. Computation, Modeling, and Simulation

Support: Science Foundation Ireland Centre for Research Training in Digitally-Enhanced Reality (d-real) Grant No. 18/CRT/6224

Title: Eeg microstates via deepcrostates: a web-based tool for accessible and scalable whole brain analysis

Authors: *A. CHIKKANKOD, S. DELANY;
Sch. of Computer Sci., Technological Univ. Dublin, Dublin, Ireland

Abstract: EEG microstates—transient yet stable patterns of neuronal activity—are a widely used method for analysing the brain's spatiotemporal dynamics. As fundamental units of cognition, microstates offer valuable insights into neural processes and contribute to our understanding of mental states, cognitive functions, and neurological disorders.Several tools exist for EEG microstate analysis, including Cartool, EEGLAB/MATLAB plugins, and Pycrostates. Each offers unique strengths but also poses limitations. Cartool, while offering rich visualisations, is platform-dependent and primarily Windows-based. EEGLAB/MATLAB plugins provide a powerful toolbox ecosystem but require a costly MATLAB license and have a steep learning curve. Pycrostates, an open-source Python implementation, is easy to install and reproducible, but assumes familiarity with programming.To address these limitations, we present Deepcrostates, a browser-accessible web application for EEG microstate analysis. This platform-independent tool requires no programming knowledge or licensed software. By eliminating barriers related to operating systems, cost, and technical expertise, Deepcrostates makes microstate analysis as accessible as any modern web application.Unlike existing tools that rely on traditional clustering methods—mainly a modified k-means algorithm dating back to 1995—Deepcrostates adopts a deep clustering approach. It combines adaptive peak detection and temporal smoothing for Global Field Power (GFP) segmentation with a convolutional autoencoder-based clustering framework. The loss function is designed to jointly optimise EEG signal reconstruction, mean correlation, and Global Explained Variance (GEV).We benchmarked

Deepcrostates against Pycrostates using two resting-state EEG datasets—LEMON and test-retest—each with six participants as proof-of-concept. Preliminary results show that Deepcrostates achieve at least 30% improvement across clustering metrics (silhouette score, Davies-Bouldin index, Calinski-Harabasz index, Dunn index) and at least 10% improvement in microstate indicators (GEV and mean absolute correlation). By combining improved analytical performance with web-based accessibility, Deepcrostates represent a significant advancement in EEG microstate analysis. It democratises access to advanced tools and enables broader, scalable use of microstates in investigating whole-brain dynamics.

Disclosures: A. Chikkankod: None. **S. Delany:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR097.18/OO17

Topic: J.06. Computation, Modeling, and Simulation

Support: NIH Grant R01MH123610
NSF Grant 2112455

Title: Dynamic Convergence and Divergence of Brain Connectivity States as Biomarkers in Psychiatric Disorders: Insights from Resting-State fMRI

Authors: N. SOLEIMANI[1], *V. CALHOUN[2];
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Abstract: Traditional static analyses of brain connectivity typically fail to capture transient neural dynamics critical for understanding psychiatric disorders. To overcome this limitation, we developed a novel analytical framework called "dynamic convergence and divergence analysis," designed explicitly to measure how the strength and similarity of brain connectivity patterns fluctuate over time. Our approach begins by using resting-state functional MRI data from a large-scale multisite dataset (N > 5k) to estimate 15 distinct "states." We performed a calibration step by standardizing each individual's connectivity data through regression onto these common population-derived states. This process generates standardized "mixing matrices," allowing us to directly compare individuals and groups without bias from inter-subject variability.Next, we calculated the Euclidean distance between the amplitudes (activation levels) of each state at each time point. Smaller distances indicate similar strengths across states, representing periods of "convergence," or network stability. Larger distances reflect differences in state strengths, marking periods of "divergence," or network variability.Applying this method to psychiatric populations, we examined connectivity dynamics in patients with schizophrenia, autism spectrum disorder, bipolar disorder, major depressive disorder, and healthy control individuals. Our results revealed disorder-specific connectivity profiles: schizophrenia patients exhibited

widespread instability across many networks; individuals with autism showed enhanced stability in specific networks; bipolar disorder patients had selective instability within emotion-related brain regions; and those with major depressive disorder displayed a mix of stable and unstable connectivity across cognitive and self-referential networks.By explicitly capturing these transient and complex shifts in connectivity, our dynamic framework provides data-driven biomarkers of neural flexibility.

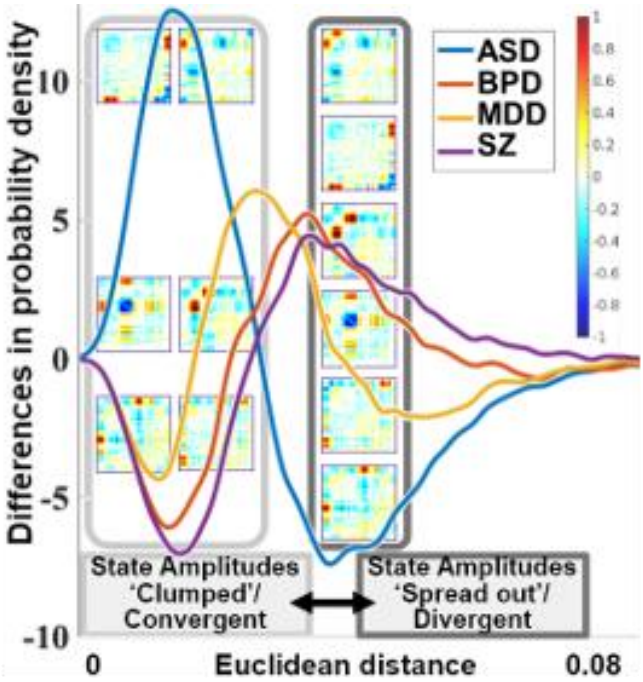


Figure 1. The distribution of pairwise Euclidean distances for schizophrenia (SCZ), autism spectrum disorder (ASD), bipolar disorder (BPD), major depressive disorder (MDD) relative to healthy controls (HC), illustrating the variability in connectivity patterns across groups. This provides a threshold-free visualization of state amplitude divergence, capturing differences in connectivity without applying predefined cutoffs.

Disclosures: N. Soleimani: None. **V. Calhoun:** None.

Poster

PSTR097: Computational Approaches in fMRI or EEG I

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Program #/Poster #: PSTR097.19/OO18

Topic: I.04. Executive Functions

Support: NSF

Title: Developing and applying a computationally tractable model to estimate higher-order correlations in fMRI data

Authors: *T. PEPPER[1], J. R. MANNING[3], L. L. OWEN[2];
[2]Psychological and Brain sciences, [1]Univ. of Montana, Missoula, MT; [3]Dartmouth Univ., West Lebanon, NH

Abstract: To better understand dynamic interactions among brain regions, we have extended **Timecorr**, our open-source analytical tool for estimating higher-order correlations, to incorporate multiple kernel functions (e.g. Gaussian, Laplacian, Mexican hat) and multiple dimensionality-reduction techniques (e.g. PCA, ICA, sparse methods) within its dynamic-correlation estimator. We conducted an extensive grid search with 3,600 hyperparameter combinations spanning kernel widths, dimensionality-reduction techniques, and kernel functions to identify settings that maximize decoding performance. Using a subset of the Simony et al. (2016) story-listening dataset in which participants were told a story under different listening conditions (intact, paragraph-scrambled, word-scrambled, and rest), we trained cross-participant classifiers with an optimized weighted blend of inferred higher-order calculations (up to 10th order). Classifiers leveraging our best-performing kernel and dimensionality-reduction settings achieved up to 50% decoding accuracy for the intact-story condition, double the accuracy obtained in our previous work. Furthermore, we demonstrate that up to 5th-order correlations capture critical narrative-driven neural interactions. By contrast, for both scrambled conditions, classifiers showed that first-order correlations or raw activation outperformed those using higher-order features, confirming that high-order network interactions are most informative when narrative structure remains intact. Moving forward, we are applying these optimized Timecorr pipelines to additional fMRI datasets to test generalization. By systematically evaluating kernel choice and dimensionality-reduction parameters across diverse paradigms, we aim to uncover stable, high-order network signatures that support complex cognition.

Disclosures: T. Pepper: None. **J.R. Manning:** None. **L.L. Owen:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.01/PP1

Topic: J.07. Data Analysis and Statistics

Title: Unifying equivalences in imaging and network neuroscience

Authors: *M. RUBINOV;
Vanderbilt Univ., Nashville, TN

Abstract: Modern neuroscience faces the challenge of integrating a wealth of data, analyses, and results. We recently showed that a neglect of this integration can lead to circular analyses and redundant explanations. Here, we help advance neuroscientific integration by describing equivalences that unify diverse analyses of neuroscientific datasets and networks. First, we equate variants of first-component removal across diverse scientific fields. We build on these

equivalences to unify *k* -means and modularity — two fundamental objectives in unsupervised learning and network science. Second, and correspondingly, we unify Lloyd and Louvain methods — two classic algorithms for data clustering and module detection. We show that our unified Loyvain method does *k* -means and spectral clustering better than standard methods on example neuroscience data. Third, we combine these results with existing knowledge to use our method for detection of general binary-valued components. As an example, we extend the method to bipartite networks and use it to do binary canonical covariance analysis. Fourth, we adopt the number of shared common neighbors, or co-neighbors, as a simple type of connectional similarity. We show that the components or modules of co-neighbor networks equate with standard or binary co-activity gradients in imaging neuroscience. Fifth, we describe the second degree, a measure of connectional dispersion that complements the (first) degree, a measure of connectional magnitude. We show that the degree and the second degree, separately and in combination, equate with six measures of communication, control, and diversity in network science. Sixth, we describe three semi-analytical vignettes that clarify and simplify the interpretation of primary cortical gradients, network growth, and dynamic node-module affinity in imaging and network neuroscience. Finally, we introduce *abct*, a multi-language toolbox that implements our analyses, and includes companion notebooks that show these analyses on example data. Together, our study describes equivalences that unify diverse analyses across unsupervised learning, network science, imaging neuroscience, and network neuroscience.

Disclosures: M. Rubinov: None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.02/PP2

Topic: J.07. Data Analysis and Statistics

Support: U54DA049110-05

Title: Image processing in the Acute to Chronic Pain Signatures (A2CPS) project

Authors: *P. SADIL;
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Abstract: Chronic pain affects 50 million Americans and has a higher incidence of new cases than many common conditions like diabetes, depression, and high blood pressure. For most people, pain due to tissue damage decreases as the injury heals, but sometimes the pain of an injury, surgery, or disease becomes chronic. The lack of effective, non-addictive treatments for chronic pain contributed to the ongoing opioid overdose crisis and is now recognized as a public health crisis in itself. The causes, risk factors, and mechanisms of chronic pain remain unclear, in part because biomarkers for pain do not exist. Finding objective readouts of the risk for and progression of pain would be a major step toward understanding and treating chronic pain.

The US National Institutes of Health Common Fund, under the Office of the Director, initiated the Acute to Chronic Pain Signatures (A2CPS) project, a multi-disciplinary endeavor to identify biomarkers and biosignatures of pain after surgery. Surgery serves as a tightly controlled acute injury, and after some surgeries, between 10 and 80 percent of patients may develop chronic pain. The project aims to test candidate, prospective biomarkers (univariate factors that prior evidence indicates may be related to chronic pain), assemble putative biomarkers into biosignatures (measures derived from combinations of several biomarkers), and catalyze the discovery of novel biomarkers or biosignatures. Chronic pain is a biopsychosocial disease, with numerous factors in each domain contributing to the transition to chronic pain. To capture these, A2CPS casts a wide net, collecting patient-reported outcomes, functional testing, omics modalities, and brain imaging. Here, we present a public release of A2CPS neuroimaging. The A2CPS project will include over 2500 participants undergoing either knee replacement or thoracic surgery. Participants are scanned before surgery, and a subset are scanned again 3 months after surgery, when chronic pain can be diagnosed. The A2CPS imaging protocol includes structural MRI, diffusion MRI, resting-state functional MRI, and tonic cuff pain functional MRI. The data have undergone extensive quality control, and derivatives have been generated to facilitate rapid biomarker evaluation, as well as general analyses.

Disclosures: P. Sadil: None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Program #/Poster #: PSTR098.03/PP3

Topic: J.07. Data Analysis and Statistics

Support: National Research Foundation of Korea RS-2023-00208884
National Research Foundation of Korea RS-2023-00265824

Title: Deep Learning-based Synthesis of Fractional Anisotropy Maps from T1-Weighted MRI for Predicting Post-Stroke Motor Outcomes

Authors: *G. KWON, H. KIM, J. LEE;
Kumoh Natl. Inst. of Technol., Gumi, Korea, Republic of

Abstract: Ischemic stroke remains a leading cause of mortality and long-term disability worldwide, underscoring the need for accurate and accessible prognostic tools to support recovery planning. While T1-weighted MRI (T1) provides detailed anatomical information, diffusion tensor imaging (DTI) captures microstructural white matter integrity via metrics such as fractional anisotropy (FA), a well-established biomarker for motor outcome prediction. However, DTI's extended acquisition time and limited clinical accessibility hinder its routine use. To address this challenge, we developed a deep learning-based framework to synthesize FA maps directly from T1-weighted MRI, thereby enabling outcome prediction without requiring

DTI acquisition. The model was trained using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI; training: n=1,121; testing: n=274), employing a U-Net-based generative adversarial network (GAN) with 2.5D input (three consecutive axial slices). For application to ischemic stroke patients (n=59), the pre-trained model was fine-tuned using six-channel inputs (T1 + b0 images) via transfer learning. Synthetic FA maps demonstrated high fidelity, showing strong agreement with real FA maps (L1 loss = 0.0053; PSNR = 32.16; SSIM = 0.9595). We evaluated three prognostic models for motor outcome at three months post-stroke: (1) T1-only, (2) T1 + real FA, and (3) T1 + synthetic FA, each combined with clinical variables. From two-week post-stroke T1 scans, lesion volume was extracted, while CST lesion load and FA values were derived from DTI. Predictive models were constructed using linear regression and evaluated via leave-one-out cross-validation (LOOCV). Results indicated that both real and synthetic FA significantly enhanced prediction accuracy. Compared to the T1-only model, the inclusion of real FA led to a 61.4% improvement, while synthetic FA achieved a 68.4% gain. When age was added as a clinical covariate, prediction gains remained robust: 66.9% with real FA and 61.7% with synthetic FA. These findings demonstrate that T1-derived synthetic FA maps can serve as reliable imaging biomarkers for stroke recovery prediction. By enabling high-accuracy FA estimation from widely available T1 scans, this approach offers a non-invasive, efficient, and scalable solution for personalized stroke prognosis and rehabilitation planning.

Disclosures: G. Kwon: None. **H. Kim:** None. **J. Lee:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

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Program #/Poster #: PSTR098.05/PP4

Topic: J.07. Data Analysis and Statistics

Title: Early Prediction of Neurodegenerative Parkinsonism via Machine Learning Integration of Semi-Quantitative SPECT Biomarkers

Authors: V. WU[1,2], J.-E. DING[1], C.-C. HSU[3], *F. LIU[1];
[1]Dept. of Systems Engin., Stevens Inst. of Technol., Hoboken, NJ; [2]The Harker Sch., San Jose, CA; [3]Dept. of Nuclear Med., Kaohsiung Chang Gung Mem. Hosp., Kaohsiung, Taiwan

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders worldwide. In particular, abnormalities in dopamine metabolism frequently occur during the progressive loss of dopaminergic neurons in the substantia nigra. Dopamine transporter (DAT) imaging with single photon emission computed tomography (SPECT) can detect presynaptic dopamine neuronal dysfunction and is helpful in the differentiation between conditions with and without presynaptic dopaminergic deficit. For DAT-SPECT interpretation, the DaTQUANT package provides automatic semi-quantification that generates objective parameters to assist with image analysis. However, conventional diagnostic approaches rely on nuclear medicine specialists to perform visual interpretation of SPECT images, which not only creates a

substantial burden on human resources but also carries the risk of misdiagnosis. In this study, we collected data from three subtypes of neurodegenerative parkinsonism (i.e., healthy controls, mild PD, and PD), including 514 patients who underwent Tc-99m TRODAT SPECT. TRODAT SPECT images were acquired using a hybrid SPECT system (Symbia T, Siemens Medical Solution). SPECT images were obtained at 30 seconds per step, acquiring 120 projections over a circular 360-degree rotation using low-energy, high-resolution parallel-hole collimators. After reconstruction with CT attenuation correction, the SPECT images were imported into DaTQUANT for automatic semi-quantification, yielding twelve semi-quantification parameters. We employed machine learning algorithms to evaluate early-stage PD classification tasks. The results demonstrated that XGBoost achieved an Area Under the Curve (AUC) of 71%, an accuracy of 69%, a precision of 71%, a recall of 68%, and an F1-score of 69% for the classification of early-stage PD as a neurodegenerative disorder. Our findings demonstrate that the specific binding ratios (SBRs) of the right and left anterior putamen (AP-R and AP-L) exhibit the characteristic asymmetric dopaminergic denervation pattern pathognomonic of PD, with unilateral predominance of neuronal degeneration. The putamen/caudate ratio (P/C ratio) reflecting regional differences in striatal areas indirectly indicates the differential degrees of impairment between the putamen and caudate during disease progression, providing clinically significant biomarkers of paramount importance for precise PD diagnosis.

Disclosures: V. Wu: None. **J. Ding:** None. **C. Hsu:** None. **F. Liu:** None.

Poster

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Program #/Poster #: PSTR098.06/PP5

Topic: J.07. Data Analysis and Statistics

Support: National Research Foundation of Korea RS-2023-00208884
National Research Foundation of Korea RS-2023-00265824

Title: Integrating DTI Metrics to Explain Functional Decline in Older Adults Using a PCA-based Approach

Authors: *J. LEE, **G. KWON**, H. KIM, W. HAN, H. KIM;
Kumoh Natl. Inst. of Technol., Gumi, Korea, Republic of

Abstract: As the global population continues to age, the increasing prevalence of cognitive and physical decline poses growing challenges for individuals and healthcare systems alike. Accurate and efficient assessment of these declines is essential for timely intervention and care planning. However, conventional assessment methods are often subjective, time-consuming, and vulnerable to external influences such as fatigue or motivation, which limits their reliability. These limitations underscore the urgent need for objective and scalable tools to evaluate age-related functional decline. Neuroimaging biomarkers have emerged as promising candidates for

such tools. In particular, diffusion tensor imaging (DTI), a non-invasive technique that captures microstructural integrity of white matter, provides several quantitative metrics that may reflect functional status. This study explores the associations between DTI-derived metrics and both cognitive and physical functions in older adults. Four key diffusion metrics-fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD)-were analyzed for their explanatory power in relation to functional outcomes. To improve the sensitivity of the analysis, principal component analysis (PCA) was employed to integrate these metrics into composite components that better capture shared variance and underlying patterns across white matter features. Results showed that neuroimaging biomarkers are effective in explaining variations in both cognitive and physical domains. While some white matter pathways were commonly associated with both domains, others were selectively related to specific subdomains, such as executive function or mobility. Notably, the PCA-based approach enhanced explanatory power compared to individual metrics alone. These findings support the utility of DTI-based biomarkers as objective tools for assessing age-related decline and suggest that integrating multiple neuroimaging features can improve early detection and personalized intervention strategies. This study highlights the potential of advanced neuroimaging in both research and clinical contexts related to aging.

Disclosures: J. Lee: None. **G. Kwon:** None. **H. Kim:** None. **W. Han:** None. **H. Kim:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Support: NRSA F32MH140418-01
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NIH R01MH060974
NIH U19AG073585
NIH S10OD025200
NIH 1S10RR022984-01A1
NIH 1S10OD018091-01

Title: Cortical Thickness Compensation for Folding-Driven Inter-Individual Variability

Authors: *N. DEMIRCI[1], T. COALSON[2], M. HOLLAND[5], D. C. VAN ESSEN[3], M. F. GLASSER[4];
[2]Neurosci., [1]Washington Univ. in St. Louis Sch. of Med., St. Louis, MO; [3]Neurosci., Washington Univ. in St. Louis Sch. of Med., St Louis, MO; [4]Radiology, Neuroscience, Biomed. Engin., Washington Univ. in St. Louis Sch. of Med., Saint Louis, MO; [5]Aerospace and Mechanical Engin., Univ. of Notre Dame, Notre Dame, IN

Abstract: Cortical thickness is a widely used neuroimaging metric to assess brain morphology and age-related cortical degeneration. However, conventional measures are confounded by cortical folding, as sulci and gyri introduce inter- and intra-individual thickness variability unrelated to neurobiological processes. To mitigate this issue, studies often apply extensive spatial smoothing (mean 24 mm FWHM), which compromises spatial precision. We introduce a novel folding-compensated cortical thickness method based on local nonlinear regression that accounts for folding-related variability without explicit smoothing. Using multiple regression across five folding descriptors (principal curvatures, Gaussian curvature, shape index, and curvedness), we estimate cortical thickness as if folding effects were minimized. The method was applied to the Human Connectome Project Young Adult (HCP-YA; N=1071, ages 22-35, 485M, 586F) and Aging (HCA; N=1798, ages 36-103, 520M, 681F) cohorts, and is integrated into the HCP processing pipelines. Folding-compensated cortical thickness reduces folding-related inter-individual variability, lowering the coefficient of variation from 15% to 10% and producing more homogeneous thickness patterns across gyri and sulci. Within-area variability also decreases, with the mean standard deviation reducing from 0.38 mm (uncompensated) to 0.22 mm (compensated). Importantly, this reduction does not alter biologically meaningful patterns, such as age-related atrophy, which remains highly correlated with the uncompensated measure ($r = 0.92$). The compensated measure yields larger standardized effect sizes for aging—reflected in higher R^2 and Cohen's f^2 across most cortical areas—due to reduced unmodeled variability. Associations with age are stronger in expected regions, such as the prefrontal and temporal cortices. Additionally, spatial gradients of compensated thickness align more closely with resting-state fMRI and myelin maps, suggesting more precise and anatomically consistent areal boundaries. These findings show that local nonlinear folding compensation yields a more accurate and biologically meaningful measure of cortical thickness, with broad implications for studies of development, aging, disease, and cross-species comparison. By reducing folding-related confounds without relying on spatial smoothing, this approach enhances measurement precision and may improve biomarker identification and cortical parcellation. Overall, accounting for folding-related variability more accurately captures individual differences in brain morphology in health and disease.

Disclosures: N. demirci: None. **T. Coalson:** None. **M. Holland:** None. **D.C. Van Essen:** None. **M.F. Glasser:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Program #/Poster #: PSTR098.08/PP7

Topic: J.07. Data Analysis and Statistics

Support: UNAM-PAPIIT TA101224

Title: From satellites to hospitals: utilizing machine learning for combined motion-correction and super-resolution in diffusion-weighted MRI of the brain

Authors: *M. J. FAIR[1,2], J. SOLÍS-PÉREZ[1], L. CONCHA[2], M. GARCIA-GOMAR[1];
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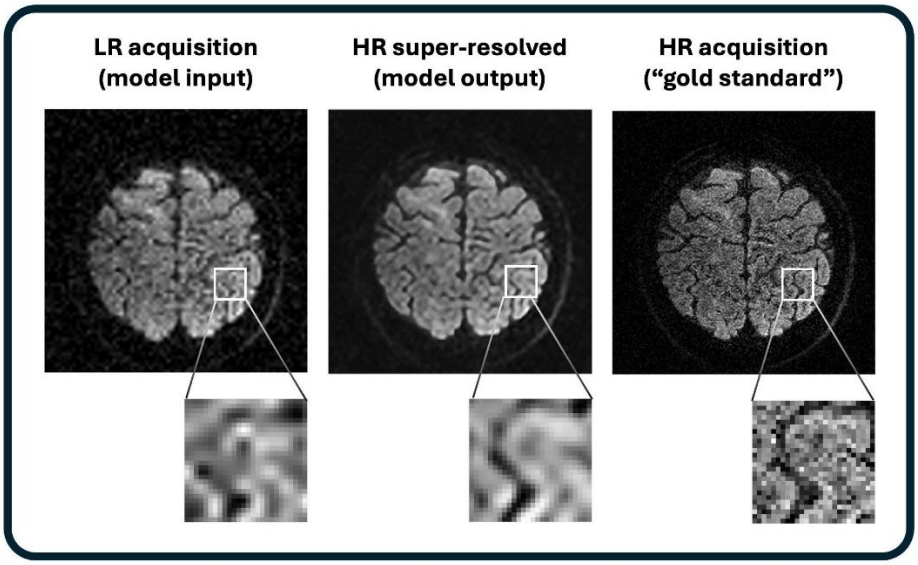
Abstract: Supervised machine learning has been widely applied in different fields of image processing, including for enhanced neuroimaging. In diffusion-weighted imaging (DWI) MRI, low spatial resolution and artefacts - such as motion errors and eddy current shifts - can impact analysis. Conventional post-processing methods attempt to address issues separately and are often slow. Machine learning offers a fast, automated approach to jointly correct artefacts, without requiring acquisition metadata, and to improve general image quality. Here, we adapt DeepSUM (Molini et al., 2019)—originally developed for satellite image registration and super-resolution—for use in neuro DWI.

DeepSUM is a convolutional neural network with three stages: a 2D super-resolution module for feature extraction, a registration network to estimate filters, and a 3D fusion network. It was adapted to take repeated DWIs as input and produce a single registered, higher-resolution image. Two models were trained: 1. on open access high resolution DWI (Wang et al., 2021) with added simulated motion (192 images) and 2. on IRB approved prospectively acquired DWI in healthy controls (360 images). Data were split into training, validation, and test sets (60% : 20% : 20%). Results are reported on the test set.

The model successfully generated high resolution (HR) outputs (Model 1: 0.8 x 0.8 mm^2, Model 2: 1.0 x 1.0 mm^2) from multiple lower resolution (LR) DWI inputs (Model 1: 2.4 x 2.4 mm^2, Model 2: 3.0 x 3.0 mm^2). On synthetic data (rigid, in plane motion) it achieved an average of (50.8 ± 11.9) % improvement in SSIM comparative to the LR images and an SNR improvement of (435 ± 50) % over the single HR acquisitions. On real data, despite more complex motion, there is visible recovery of fine details and similar SNR gains.

The model leverages motion across DWI acquisitions - traditionally a drawback - to enhance spatial resolution and image quality. Ongoing work aims to improve robustness and applicability to variations in the input data.

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Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Program #/Poster #: PSTR098.09/PP8

Topic: J.03. Anatomical Methods

Support: National Science Foundation of China (No. 82371486)

Title: Parcellation of individual brains: From group level atlas to precise mapping

Authors: *Y. CUI, C. LI, S. YU;
Chinese Acad. of Sci. Inst. of Automation, Beijing, China

Abstract: Accurate mapping of brain functional regions at the individual level is crucial for understanding variations in brain function and behavior, identifying brain abnormalities, and developing personalized treatments for neuropsychiatric disorders. While most research focuses on a single modality of data, integrating different data modalities may provide complementary information and improve the accuracy of individual brain parcellation.
We propose two novel methods for individual brain parcellation: multimodal connectivity-based individual parcellation (MCIP) and a deep learning pipeline for multimodal subject-specific parcellation with task contrasts synthesis (TS-AI). MCIP optimizes within-region homogeneity, spatial continuity, and similarity to a reference atlas by fusing personal rsfMRI- and dMRI-derived connectivity. As a model-free method, MCIP can be directly applied to individual subjects without training a model, and is suitable for patients with brain abnormalities or animals. TS-AI is a model-based method that captures high-order and nonlinear relationships between individual-specific information and individual parcellation. TS-AI enables the synthesis of task contrast maps without the need for actual task fMRI scans, and a feature consistency loss is designed to mitigate overfitting risks caused by the absence of ground truth for model-based individual parcellation.
To assess the performance of MCIP- and TS-AI-derived individual parcellations, test-retest reliability, parcellation-based behavior prediction performance, as well as anatomical connectivity, resting-state functional, and task activation homogeneities were evaluated. Both MCIP and TS-AI demonstrated better parcellation performance compared to the multimodal individual parcellation method [1]. For MCIP, comparative investigations were further conducted between humans and macaques, revealing higher topographic variability in humans than in macaques. For TS-AI, sensitivity analysis provided insights into region-specific features influencing individual variation in functional regionalization. TS-AI also identified accelerated shrinkage in the medial temporal and frontal parcels during the progression of Alzheimer's disease.
The validations of MCIP and TS-AI highlight the importance of individualized brain mapping

for understanding brain function, behavior, and neuropsychiatric disorders, offering potential applications in precision medicine, particularly personalized diagnosis and treatment.
References: [1] Glasser, M.F., et al., 2016. A multi-modal parcellation of human cerebral cortex. Nature 536, 171-178.

Disclosures: Y. Cui: None. **C. Li:** None. **S. Yu:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Program #/Poster #: PSTR098.10/PP9

Topic: J.07. Data Analysis and Statistics

Title: White matter connectivity reveals horizontal-vertical and vertical-meridian asymmetries in the visual system

Authors: *G. AMOROSINO[1], J. KWON[1], M. CARRASCO[2], B. CARON[1], F. PESTILLI[1]; [1]The Univ. of Texas at Austin, Austin, TX; [2]NYU, New York, NY

Abstract: Introduction:
Visual performance shows horizontal-vertical (HVA) and vertical-meridian (VMA) asymmetries: acuity, contrast sensitivity, and resolution are better along the horizontal than vertical meridians, and better on the lower than upper vertical meridian. While cortical asymmetries are known, analogous patterns in white matter (WM) connectivity are unexplored. We examined HVA and VMA in WM connectivity using diffusion MRI data from 1,800 subjects from HCP, PING, and Cam-CAN.
Methods:
We developed a processing pipeline deployed on brainlife.io computation platform, combining tractography and automated population receptive field mapping. Cortical surfaces were divided into polar angle meridians (horizontal, vertical, diagonal) and iso-eccentricity bins (0-2°, 2-4°, 4-6°, 6-8°). Streamlines were classified as intra-occipital (endpoints within the same wedge) or long-range (one endpoint outside the occipital lobe). Twelve visual areas (V1, V2, V3, V3a, V3b, hV4, LO1, LO2, TO1, TO2, VO1, VO2) were defined per wedge. Streamline density was calculated by dividing the streamline count by the average region volume. Repeated-measures ANOVA tested asymmetries, followed by post hoc t-tests.
Results:
Intra-occipital connections showed higher density along the horizontal than vertical meridians (HVA) and higher density on the lower versus upper vertical meridian (VMA) (Figure 1a). Long-range connections showed significant HVA but no VMA (Figure 1b). Asymmetries were consistent across visual areas and cohorts.
Conclusions:
We reveal HVA and VMA in visual WM connectivity, aligning structural patterns with perceptual asymmetries. Robust effects across subjects and regions suggest an inherent WM

architecture shaping visual field performance. Our open-source pipeline provides a reproducible framework to study the visual connectome and underscores WM's role in perceptual asymmetries.

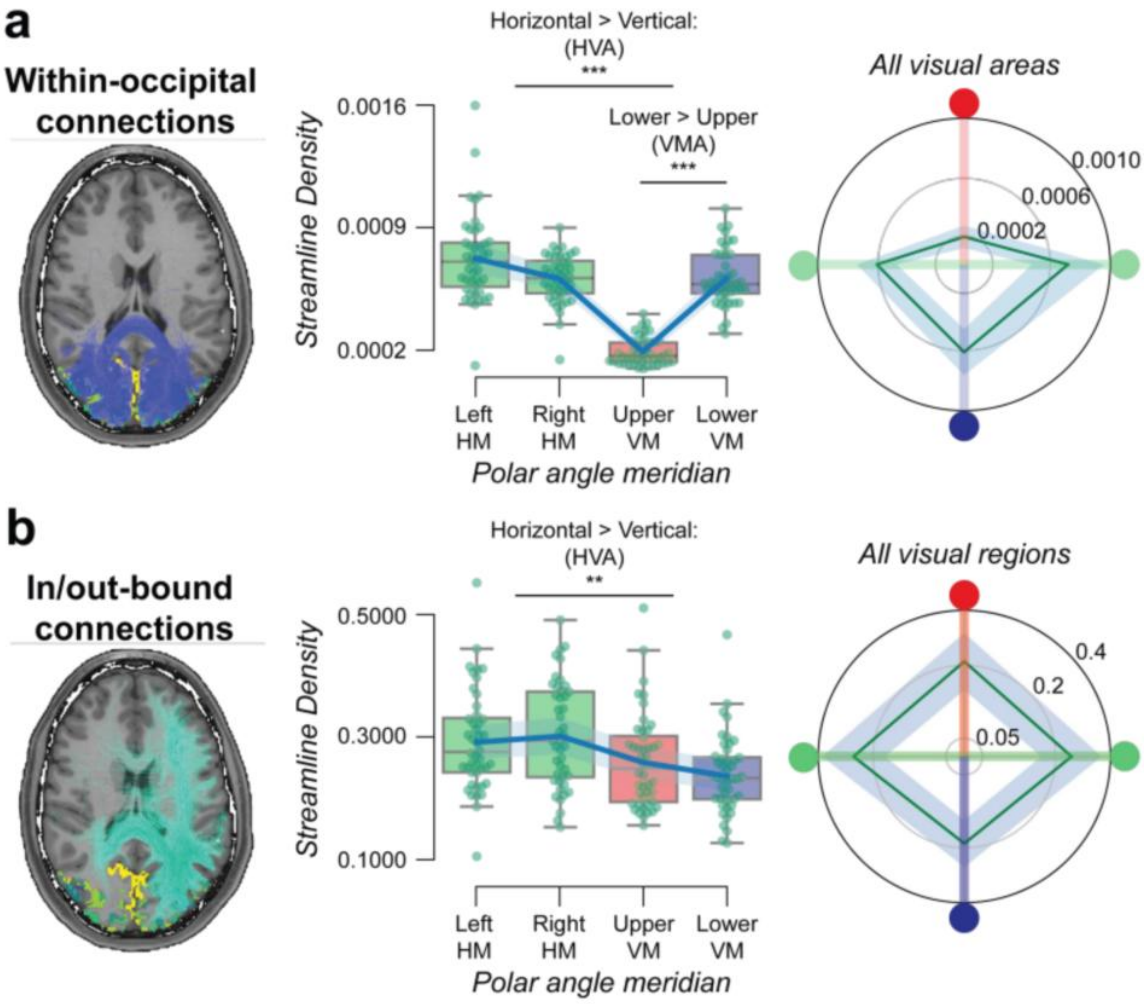


*Figure 2. a) Within-occipital conncetions. b) In/out-bound connections. First column: visualization of tracts. Second column: Average streamline density within polar angle meridians. Box and whisker plots denote the median (solid line), 25th and 75th quartiles (box), and 0th and 100th quartiles (whiskers). Individual participant data points are overlaid (green dots). Third column: Spyder plot denoting the average streamline density within each polar angle meridian (green line), with standard deviation (shaded blue error bars). *** $p < 0.001$. ** $p < 0.01$.*

Disclosures: G. Amorosino: None. **J. Kwon:** None. **M. Carrasco:** None. **B. Caron:** None. **F. Pestilli:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.11/PP10

Topic: J.07. Data Analysis and Statistics

Support: National Eye Institute R01 EY033628

Title: Hybrid 2D/3D convolutional neural networks can automatically segment functional regions of the visual cortex

Authors: *Q. LI[1], N. C. BENSON[2];
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Abstract: The visual cortex is tiled with retinotopic maps, and most contemporary visual neuroscience studies require an accurate segmentation of at least some of these maps. Segmenting the visual cortex into functional regions, however, requires that human researchers invest substantial time and expertise in collecting and annotating visual experiments, which are typically expensive. Visual area boundaries can be predicted using anatomical alignment to an atlas, but these anatomically-derived predictions are less accurate than the boundaries drawn by human researchers. Recent research using convolutional neural networks (CNNs) to automatically segment V1, V2, and V3 based on 2D images of functional data projected onto a flattened cortical surface have demonstrated that CNNs are capable of segmenting early visual areas with the same accuracy as humans (Benson et al., 2025, DOI:10.1101/2025.05.16.654503). However, functional maps are much more difficult to collect than structural images such as T1-weighted and diffusion-weighted images, and the ability to segment the brain's functional regions using structural data alone would benefit many research groups. In particular, diffusion-weighted data are inherently 3D and cannot easily be adapted to 2D CNNs. Here, we first built a 3D CNN capable of exploiting 3D data such as diffusion-weighted images, and although its performance was not as high as a 2D CNN due to the additional complexities of 3D data, its performance was comparable with existing anatomical template methods when provided with T1-weighted data and was significantly improved by diffusion-weighted data. Because the segmentation problem is substantially harder in 3D than in 2D, we additionally built a hybrid 2D/3D CNN for visual cortex segmentation by combining 2D and 3D U-net CNNs with ResNet backbones (Ronneberger et al., 2015, DOI:10.48550/arXiv.1505.04597; Razman et al., 2020, DOI:10.1007/s10916-019-1475-2). The 3D CNN extracts volumetric feature maps from the 3D MR images, which are then projected onto the 2D cortical surface using a sparse transformation matrix. These projections are then stacked with other 2D surface features and passed through the 2D U-net, which produces predictions of the boundaries on the cortical surface. The 3D and 2D CNNs are trained simultaneously as a single model with a unified set of parameters, allowing the 3D CNN to learn the features that are most useful to the 2D CNN. The ability to combine 2D cortical surface properties and alignments with 3D data extracted from MR images is a valuable tool for future neuroimaging work.

Disclosures: Q. Li: None. **N.C. Benson:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.12/PP11

Topic: J.07. Data Analysis and Statistics

Title: Trends-setter: a unified containerized platform for reproducible bids-validated neuroimaging workflows

Authors: *H. BOCKHOLT, B. T. BAKER, V. CALHOUN;
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Abstract: Reproducible neuroimaging workflows are impeded by heterogeneous software stacks, complex installations, and version conflicts. Although BIDS (Brain Imaging Data Structure) standardizes data organization[1], configuring processing environments remains a barrier, diverting effort from core scientific questions.
TReNDS-Setter is a containerized neuroinformatics platform that packages and unifies: • Core suites: AFNI, FSL, SPM, ANTs, FreeSurfer, MRtrix3 • BIDS-compliant pipelines: fMRIPrep[2], sMRIPrep, QSIPrep, QSIRecon, ASLPrep • Advanced modules: GIFT Neuromark ICA for static and dynamic functional network connectivity (sFNC, dFNC), C-PAC connectivity, DIPY diffusion analytics, Brain Connectivity Toolbox (BCT) for FNCs. • Build tools: Neurodocker recipes, NeuroDebian base, Conda-managed neuroenv Python environment
A single wrapper script streamlines: 1. BIDS validation of inputs 2. Pipeline selection via intuitive flags 3. Automatic resource detection for multi-node or cloud-based execution 4. Standardized outputs of BIDS derivatives
The container uses multi-stage builds to minimize image size (~8 GB), integrates CI checks to lock tool versions, and offers a plugin API for adding lab-specific scripts. It supports both Docker and Singularity[3], enabling seamless operation across laptops, HPC clusters, and cloud platforms without modification.
Key Features: • Extensibility: Modular directory and plugin API for custom tools • Licensing: No individual/institutional license requirements • Portability: Consistent execution across environments—no local installation required • Usability: Unified interface abstracts dependency management • Reproducibility: Encapsulated tool versions and CI-tested builds ensure bit-wise consistency • Community Support: Public issue tracker, versioned releases, documentation, and example BIDS datasets • Adoption: Early adopters report ≥60 % reduction in setup time and seamless cross-site collaboration
TReNDS-Setter is open-source on GitHub under CC BY-NC 4.0. license. Pre-built Docker image, Singularity recipes, and example datasets are provided for immediate community adoption.
TReNDS-Setter delivers a turnkey, extensible container with plugin support and CI-verified builds, effectively lowering technical barriers, accelerating experimental iteration, streamlining cross-site harmonization, and advancing reproducible neuroimaging research.
References: [1] Gorgolewski et al., 2016 [2] Esteban et al., 2019 [3] Kurtzer et al., 2017

Disclosures: H. Bockholt: None. **B.T. Baker:** None. **V. Calhoun:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

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Program #/Poster #: PSTR098.13/PP12

Topic: J.07. Data Analysis and Statistics

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PID2023-149410OB-100 funded by MCIU/AEI/10.13039/501100011033/FEDER, EU

Title: Utility of across-echo connectivity as a Quality Control Metric for fMRI

Authors: *J. GONZALEZ-CASTILLO[1,2], C. CABALLERO GAUDES[2,3], D. A. HANDWERKER[4], P. BANDETTINI[4,5];
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Abstract: With BOLD fMRI, several quality assurance (QC) metrics exist (e.g., temporal signal-to-noise ratio (*TSNR*), test-retest reliability), yet their interpretation and usefulness remain challenging. For example, higher *TSNR* suggests better data, yet removal of neuronally-driven BOLD signal would also increase it. Also, perfect reliability across sessions can obscure relevant changes in functional connectivity (FC) over time. To overcome this, we propose a new QC metric for multi-echo (ME) fMRI that quantifies the likelihood of data being dominated by BOLD effects.

ME fMRI acquires data at multiple echo times (TEs). BOLD fluctuations are TE-dependent, while non-BOLD fluctuations are not. Prior work has shown that such differential TE-dependence can be used for automatic denoising. Here we show that FC behavior across TEs can help evaluate data quality and reveal noise content not evident with classical QC metrics.

Theory: Assuming mono-exponential decay and little thermal noise, the signal at a voxel x, time t, and echo time TE_i can be approximated as: $\Delta S(x,t,TE_i) \approx \Delta\rho(x,t) - \Delta R_2^*(x,t)TE_i$; where $\Delta\rho(x,t)$ denotes changes in net magnetization, and $\Delta R_2^*(x,t)$ changes in transverse relaxation. If data is non-BOLD dominated ($\Delta R_2^*(x,t)\approx 0$), the covariance (C) between regions' timecourses is TE-independent. Conversely, when data is BOLD dominated ($\Delta\rho(x,t)\approx 0$) covariance will be TE-dependent as follows: $C[S(x,TE_i),S(y,TE_j)] \approx (TE_i*TE_j)/(TE_k*TE_l)*C[S(x,TE_k), S(y,TE_l)]$. Based on this dichotomy, we propose a metric called p_{BOLD} that quantifies how well fMRI data adheres to this TE-dependent profile present only when BOLD dominates.

Methods: To confirm these theoretic observations, we analyzed 436 ME scans (Neurocognitive Aging Dataset). Data was pre-processed in different ways: with/without NORDIC, with/without global signal regression (*GSR*), and with/without tedana. For all pipelines we computed both p_{BOLD} and *TSNR*.

Results: Without *NORDIC*, p_{BOLD} shows a data quality increase following tedana denoising. Yet, with GSR, p_{BOLD} shows a decrease in quality. Next, *NORDIC*+tedana denoising results in higher

TSNR, but lower p_{BOLD}.
Conclusions: p_{BOLD} alerts to lower data quality in cases where *TSNR* suggests otherwise. For *NORDIC+tedana*, further exploration revealed issues with component estimation after *NORDIC*. *GSR* results in less BOLD-dominated data. As *GS* contains two forms of *BOLD* fluctuations—namely physiological fluctuations (e.g., respiration, cardiac) and fluctuations associated with neural components of interest—to correctly interpret the decrease in p_{BOLD} caused by *GSR* one must consider the relative contributions of these two components to the *GS*.

Disclosures: J. Gonzalez-Castillo: None. **C. Caballero Gaudes:** None. **D.A. Handwerker:** None. **P. Bandettini:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.14/PP13

Topic: J.07. Data Analysis and Statistics

Title: A novel method for enhancing bold signal detection in resting-state and task-based fMRI

Authors: *Y.-W. SUNG[1], S. OGAWA[2];
[1]Kansei Fukushi Res. Inst, Tohoku Fukushi Univ., Sendai, Japan; [2]Tohoku Fukushi Univ., Sendai, Japan

Abstract: Resting-state functional magnetic resonance imaging (rs-fMRI) is extensively employed to investigate brain function in both health and disease. While the rs-fMRI signal is presumed to reflect the blood oxygen level-dependent (BOLD) response, isolating the true BOLD component within these signals remains a significant challenge due to the absence of an explicit reference, unlike in task-based fMRI (tb-fMRI), where task paradigms guide BOLD identification. To address this issue, we developed a novel technique termed the **BOLD-filter**, which leverages the echo-time (TE) dependency of BOLD signals to extract BOLD components from rs-fMRI data. This approach enhances the sensitivity and specificity of BOLD signal detection and is applicable to both resting-state and task-based conditions. MRI data were acquired using a 3T Skyra-fit MRI scanner (Siemens, Germany) equipped with a standard 20-channel head matrix coil. Functional data were collected using a multi-echo echo planar imaging (EPI) sequence with three echo times. For the rs-fMRI condition, thirty participants (11 males, 19 females) were scanned at rest with eyes closed. For the tb-fMRI condition, twenty participants (10 males, 10 females) viewed visual stimuli consisting of movie clips. The BOLD-filter method was evaluated against conventional preprocessing approaches by identifying brain voxels exhibiting BOLD characteristics. In rs-fMRI, BOLD features were defined by TE-dependent signal changes and coherence across echoes. In tb-fMRI, voxel-wise regression against the known stimulus paradigm was used. Results demonstrated that, in their most favorable cases, the BOLD-filter identified approximately six times more BOLD-active voxels in rs-fMRI and over eleven times more in tb-fMRI compared to traditional methods ($p = 0.001$ in both cases). These

findings underscore the BOLD-filter's potential to significantly improve BOLD signal detection, thus enabling more accurate and sensitive characterization of brain activity in both resting and task-engaged states. This methodological advance holds promise for broad applications in both basic neuroscience and clinical research.

Disclosures: Y. Sung: None. **S. Ogawa:** None.

Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR098.15/PP14

Topic: J.07. Data Analysis and Statistics

Title: Estimating directed brain connectivity from resting-state fMRI and structural priors using network diffusion models

Authors: *F. ARAB, A. RAJ;
Univ. of California San Francisco, San Francisco, CA

Abstract: Understanding how information flows through the brain requires identifying directional interactions between regions. While resting-state fMRI provides rich temporal dynamics, it is often reduced to symmetric functional connectivity (FC), masking the direction of neural communication. To infer directionality, methods like Granger causality, Dynamic Causal Modeling (DCM), and constraint-based discovery algorithms have been proposed. However, each has limitations: Granger causality is sensitive to noise and cannot capture contemporaneous effects, critical in fMRI's low temporal resolution; DCM, even in efficient variants, produces results difficult to interpret, often lacking statistical significance and biological interpretability; and constraint-based approaches assume acyclicity, scale poorly with network size, and typically disregard anatomical information. A major drawback of these methods is their failure to constrain inferred directional edges to follow structural connectivity (SC) or incorporate SC weights, leading to biologically implausible estimates. To address these limitations, we propose a framework that estimates biologically plausible directed connectivity by integrating SC and resting-state fMRI data within a Network Diffusion Model (NDM). Prior studies show that NDMs capture large-scale FC patterns via diffusion over SC. We extend this framework to estimate subject-specific directed connectivity matrices that best explain empirical FC patterns while remaining structurally grounded.
Unlike traditional NDMs, which use symmetric graph Laplacians from SC, our model learns an asymmetric diffusion operator. We constrain the directed connectivity matrix to be a subset of the subject's SC and penalize deviations from SC weights. This regularization ensures the sum of reciprocal connection weights stays close to the original SC while allowing asymmetry. We formulate this as an optimization problem and perform grid search over hyperparameters using data from 100 Human Connectome Project (HCP) subjects. The optimal hyperparameters are then used to estimate directed connectivity for an independent cohort of 600 additional subjects.

This results in personalized, directed graphs grounded in anatomy and supported by functional evidence. At the group level, we find consistent motifs, including source regions in control and default mode networks, and sink regions in limbic areas. Our findings show that diffusion-based models can uncover biologically meaningful directionality in resting-state brain networks, offering a scalable framework for studying directional connectivity in large populations.

Disclosures: F. Arab: None. **A. Raj:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

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Program #/Poster #: PSTR098.16/PP15

Topic: J.07. Data Analysis and Statistics

Title: Alterations of Brain Synergistic and Redundant Interactions in Patients with Parkinson's Disease and Atypical Parkinsonian Disorders

Authors: *H. LYU[1,2], W. YIN[2], W. JIANG[2], Y. LU[3], W. LIN[2];
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Abstract: Parkinson's disease (PD) and atypical parkinsonian disorders (APD) - including multiple system atrophy (MSA; cerebellar [MSA-C], parkinsonian [MSA-P], and combined [MSA-PC] phenotypes) and progressive supranuclear palsy (PSP) - are neurodegenerative diseases marked by motor and non-motor symptoms due to widespread brain network dysfunctions. While traditional neuroimaging focuses on structural changes, recent approaches emphasize the inter-regional interactions via synergistic and redundant information processing. Synergy reflects integration of complementary information; redundancy indicates overlapping or duplicated information among pairs of brain regions. Alterations in these features may signal disruptions in network efficiency, flexibility, and resilience. This study examined whole brain and network-level synergy and redundancy in PD, MSA subtypes, and PSP, compared to healthy controls (HC). We included 430 participants: 89 PD, 95 MSA-C, 74 MSA-P, 50 MSA-PC, 38 PSP patients, and 84 HC. All underwent resting-state fMRI and T1 MRI, processed using fMRIPrep and XCP-D. Partial information decomposition was applied to parcellated timeseries (Schaefer 200 atlas + 56 subcortical regions) to compute normalized synergy and redundancy for each subject. We found that synergy was significantly elevated in PD, MSA and PSP compared to HC, while redundancy was significantly reduced, after controlling for age, sex and motion (FDR corrected $p<0.05$). At the network level, sensorimotor, salience, and limbic networks, along with subcortical regions, thalamus and hippocampus, showed increased synergy and reduced redundancy in PD and APD relative to HC (FDR corrected $p<0.05$). Notably, MSA-C and MSA-PC showed a distinct pattern of reduced synergy alongside elevated redundancy in the cerebellum (FDR corrected $p<0.0001$) - an effect not observed in PD, MSA-P, or HC. While

increased synergy and decreased redundancy in the salience network and hippocampus were present in most disease groups, the alterations were less remarkable in MSA-C. Elevated synergy and reduced redundancy in PD, MSA, and PSP suggest a shift toward more integrative processing, potentially indicating the presence of functional compensation resulting from neurodegenerative damage. However, the unique reversal of this pattern in the cerebellum of MSA-C and MSA-PC - marked by decreased synergy and increased redundancy - may reflect the distinct cerebellar dominated pathology and impaired network efficiency of these subtypes.

Disclosures: H. Lyu: None. **W. Yin:** None. **W. Jiang:** None. **Y. Lu:** None. **W. Lin:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Program #/Poster #: PSTR098.17/PP16

Topic: J.07. Data Analysis and Statistics

Support: John D. and Catherine T. MacArthur Foundation to Vanderbilt University, with a subcontract to Virginia Tech
Red Gates Foundation

Title: Decoding Past Legal Mental States

Authors: *J. K. NELSON[1], L. P. SANDS, III[3], L. BARBOSA[3], T. M. LOHRENZ[3], O. JONES[4,5], G. YAFFE[6], P. MONTAGUE[3,2];
[1]Biomed. Engin., Virginia Tech., Roanoke, VA; [2]Dept. of Physics, Virginia Tech., Blacksburg, VA; [3]Fralin Biomed. Res. Inst. at VTC, Roanoke, VA; [4]Vanderbilt Law Sch., [5]Dept. of Biol. Sci., Vanderbilt Univ., Nashville, TN; [6]Yale Law Sch., Yale Univ., New Haven, CT

Abstract: Criminal convictions require not only proof of a guilty act (*actus reus*), but also of a culpable mental state (*mens rea*). In many cases, distinguishing whether a defendant acted *knowingly* or *recklessly* determines their legal culpability and the severity of punishment. Prior work (Vilares et al., 2017) showed that fMRI data collected during a task designed to simulate the knowing/reckless (K/R) distinction can successfully differentiate these mental states—but only when brain activity is recorded in the moment, as the act unfolds in the scanner. Yet in real-world legal contexts, where *mens rea* must be inferred from post hoc information, such mental states are rarely available for direct observation. For neuroimaging to have forensic relevance, we must determine if legally relevant mental states can be reconstructed from memory, after the fact. To test whether delayed memory of evidence can reveal the mental state under which an act was committed, we modified the contraband task for memory recall, assigning forty participants to either Knowing (K) or Reckless (R) conditions and conducting follow-up fMRI scans after either 3 days or 1 month. During the scan, participants viewed images from the original task (familiar stimuli) alongside novel distractors (unfamiliar stimuli). We trained linear classifiers (elastic net, logistic regression, and SVM) on fMRI responses during the memory probe and

found that responses to unfamiliar stimuli—and especially the contrast between familiar and unfamiliar—most accurately predicted K/R status (AUCs: 0.95-0.98; accuracies: 90-93%). Decoding performance remained robust across the 3-day and 1-month delays. These findings suggest that the brain's memory responses can reliably reflect whether someone acted knowingly or recklessly, even weeks after the event. Ongoing representational similarity analyses aim to quantify how memory traces evolve over time and whether this drift varies by mental state, potentially enabling distinctions between recent from remote recollection. Together, these results offer proof of principle that legally relevant mental states can leave detectable neural imprints in memory—suggesting that intent can be inferred even when the original moment of decision is no longer accessible, and offering a potential framework for inferring *mens rea* in real-world legal contexts.

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Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR098.18/PP17

Topic: J.07. Data Analysis and Statistics

Title: Sample Size-Dependent Optimal Conditioning for Pruning Non-Causal Relations in fMRI

Authors: *S. SEYEDI HASANABADI[1], F. ARAB[2], A. GHASSAMI[3], E. NOZARI[4]; [1]Mechanical Engin., UC Riverside, Riverside, CA; [2]Radiology and Biomed. Imaging, UCSF, San Francisco, CA; [3]Boston Univ., Department of Mathematics and Statistics, MA; [4]Mechanical Engin., Univ. of California, Riverside, Riverside, CA

Abstract: Accurately pruning statistical relationships and distinguishing causation from correlation, often referred to as causal discovery, is a central challenge in neuroscience. Among the many proposed methods, conditioning is one of the most widely used (Marrelec et al., 2006; Peterson et al., 2023). In short, if two variables X and Y are correlated but neither is the cause of the other, either because a third variable Z causes them both ($X \leftarrow Z \rightarrow Y$) or is the mediator between them ($X \rightarrow Z \rightarrow Y$), conditioning on Z can make X and Y independent. Thus, it is sometimes believed that conditioning on as many potential common causes and mediators as possible, leads to sparser and more accurate causal graphs. While sometimes true, this depends on the often-overlooked assumption of large (theoretically infinite) data (Kocaoglu, 2023; Marinazzo et al., 2012). How conditioning affects the accuracy of causal discovery with finite data, however, is not well understood. This is less problematic with sufficient samples (Rawls et al., 2023), but critical when data are limited compared to the size of the network, as in task fMRI.

In this work, we investigated how the available number of data samples affects the optimal size of the conditioning set in causal discovery from human fMRI. To parametrically vary the size of

conditioning set, we combined our previously proposed algorithm CaLLTiF (Arab et al., 2025), with the classical PC algorithm- as done previously in PCMCI (Runge et al., 2019). This provides us with a tunable hyperparameter α, where α = 1 and 0 result in full and no conditioning, respectively. Using several rest and task fMRI datasets (Human Connectome Project, Decoded Neurofeedback, and Microstructure Informed Connectomics), we then constructed causal graphs while parametrically varying α and the number of available samples. To assess optimality, we used the causal graph estimated from the largest available sample size as the reference graph, and for each sample size, we found the level of α that resulted in the best match with this reference.
Across all datasets and conditions, we observed a consistent pattern: the optimal value of α increased with the number of samples. That is, larger conditioning sets (higher α) resulted in more accurate graphs when sample sizes were large, whereas smaller conditioning sets (lower α) performed better for smaller sample sizes. These results provide the first characterization of the strong and monotonic relationship between the optimal size of conditioning set and length of available data in causal discovery, and support the need for sample-size-adaptive tuning of conditioning sets in causal discovery pipelines.

Disclosures: S. Seyedi HasanAbadi: None. **F. Arab:** None. **A. Ghassami:** None. **E. Nozari:** None.

Poster

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Program #/Poster #: PSTR098.19/PP18

Topic: J.07. Data Analysis and Statistics

Title: Distilling interpretable sparse trees from fMRI data: an information theory approach to understanding brain connectivity

Authors: *A. JAIN[1,2,3], M. LIN[3], A. M. D'MELLO[1,4,5];
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Abstract: Functional connectivity (FC) approaches have resulted in significant advances in the analysis of functional MRI (fMRI) data and in our understanding of human brain organization. However, FC methods typically rely on linear correlations between regions of interest (ROIs) in the brain. Thus, FC maps primarily visualize co-activation patterns in neuroimaging data, and can be difficult to interpret. In addition, methods that rely on linear correlations do not capture any complex nonlinearities in interregional communication. A method that preserves non-linearities in fMRI data could result in more interpretable and biologically-meaningful connectivity maps. Here, we leverage large-scale fMRI datasets to test such a method: using mutual information (MI) to construct sparse 'Chow-Liu' (CL) trees of ROI interactions in fMRI data. By leveraging MI, a probabilistic measure for "amount of information" shared, we predict

indirect connections in functional scans (e.g. ROIs A and B communicate through a mutual relation C). We then use these predictions to construct sparse trees of information flow via intermediate ROIs, yielding more interpretable downstream predictions than traditional, dense FC networks. We implemented the CL method in two publicly-available fMRI datasets that differed in the data modality (rest vs. task), sampling (shallow sampling in large n vs. deep-sampling in small n), and diagnostic profile (autism vs. neurotypical): ABIDE and Midnight Scan Club. Distilling sparse trees through indirect contacts, we found that CL (a) efficiently represented connectivity structures and (b) allowed for biologically interpretable visualizations. In ABIDE, using CL to predict autism from resting-state data through gaussian naive bayes regression was ~10% more accurate than FC. In MSC, using CL to map individual-level networks from task-based data recovered clusters of the same canonical brain networks as traditional FC approaches (i.e. vision, motor, attention) but with some contrasts in regional contacts and individual differences in participants' network organization. Together, our data suggest that CL is a promising approach to analyze diverse types of fMRI data, recover biologically interpretable brain organization, and confer efficiency by representing connectivity as sparse trees.

Disclosures: A. Jain: None. **M. Lin:** None. **A.M. D'Mello:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

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Program #/Poster #: PSTR098.20/PP19

Topic: J.07. Data Analysis and Statistics

Support: NIH R01 AG081655
K23 AG070320

Title: More atrophy, less antisocial behavior: inverse relationship between volume loss and behavior symptoms in patients with behavioral variant frontotemporal dementia

Authors: *B. AKBARIAN[1], K. HETT[2], T. PHAN[2], C. CONSIDINE[2], R. DARBY[2];
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Abstract: Antisocial behaviors are common in patients with behavioral variant frontotemporal dementia (bvFTD), but the neural correlates remain unclear. Antisocial behaviors in other disorders relate to abnormalities in the orbitofrontal / ventromedial prefrontal cortices (OFC/vmPFC). Here, we test whether antisocial behaviors in bvFTD relate to OFC/vmPFC atrophy. Antisocial behavior was measured using the Social Behavior Questionnaire (SBQ). First, we measured the association between OFC/vmPFC volume and antisocial behaviors in bvFTD (n=32) using linear regressions including age and sex as covariates. Next, bvFTD patients were stratified into high (n=16) and low antisocial behavior groups based on the median

SBQ score and were compared to healthy controls (n=33) using ANCOVA with age and sex as covariates. Finally, exploratory voxel-wise analyses measured associations between antisocial behavior and brain volume outside the OFC/vmPFC. Results revealed a significant positive association between brain volume and antisocial behavior (Fig 1). Interestingly, although both bvFTD groups had lower brain volume than healthy controls, patients with higher antisocial behavior had volumes closer to those of the control group (Fig 2). These findings suggest a more complex relationship between brain atrophy and behavioral symptoms in bvFTD than previously assumed, with implications for understanding brain-behavior relationships and interpreting MRI biomarkers for potential treatment response.

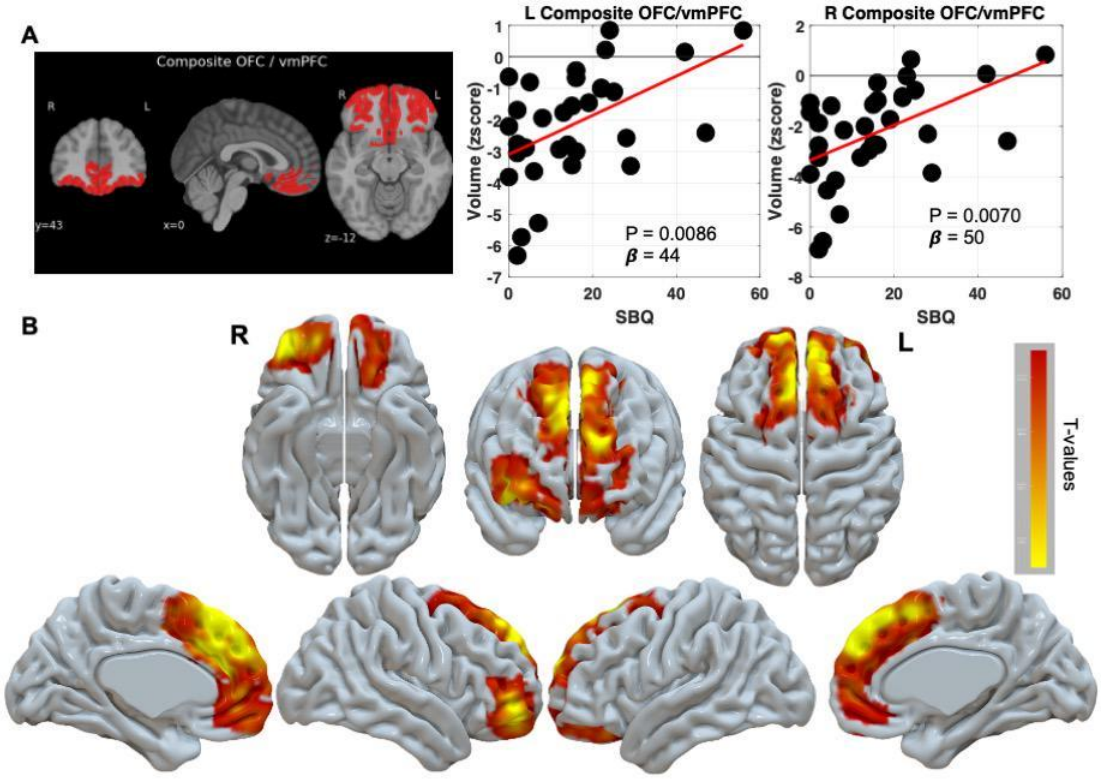


Fig 1: Increased antisocial behavior in patients with behavioral variant frontotemporal dementia is associated with higher brain volumes **(A)** within orbitofrontal and ventromedial prefrontal regions (ROI analysis), and **(B)** other related frontotemporal regions (whole-brain voxel-wise analysis).

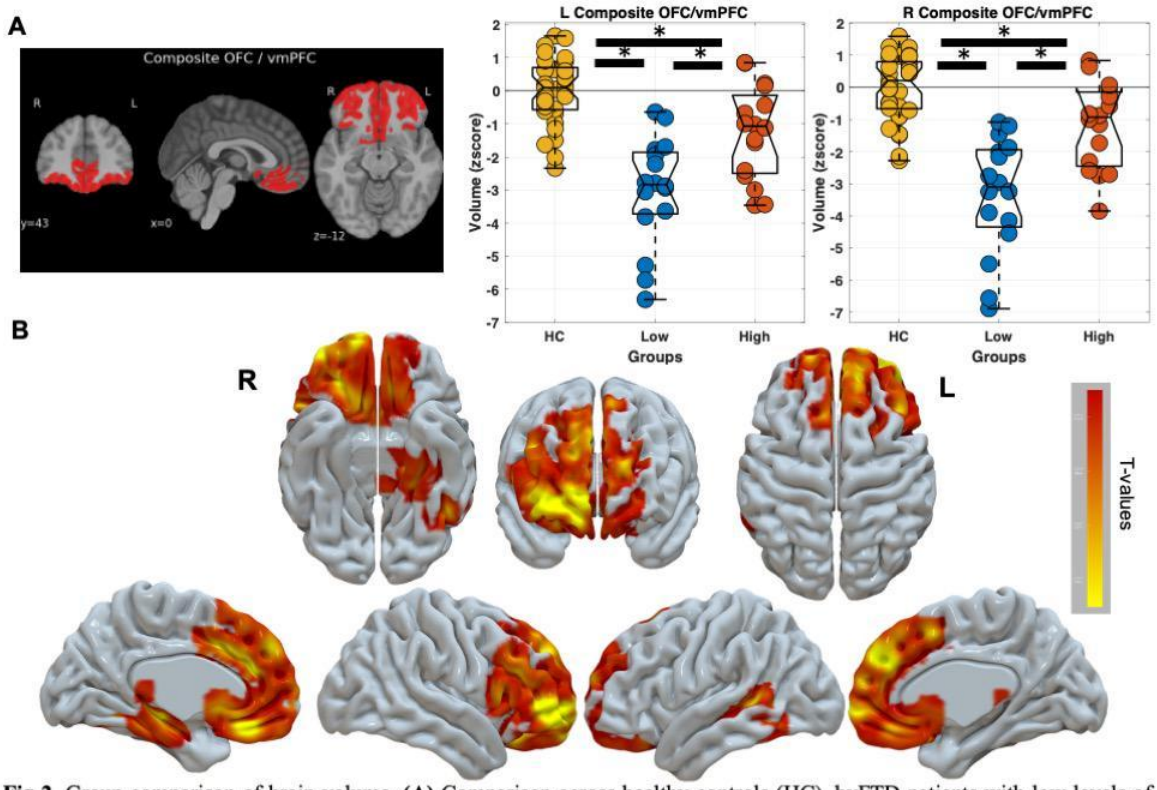


Fig 2. Group comparison of brain volume. **(A)** Comparison across healthy controls (HC), bvFTD patients with low levels of antisocial behaviour (Low), and bvFTD patients with high levels of antisocial behaviour (High) within the composite orbitofrontal/ventromedial prefrontal cortices (OFC/vmPFC) region, and **(B)** pairwise comparison of brain Low and High (whole-brain voxel-wise analysis).

Disclosures: B. Akbarian: None. **K. Hett:** None. **T. Phan:** None. **C. Considine:** None. **R. Darby:** None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.21/Web Only

Topic: J.07. Data Analysis and Statistics

Title: Meta-state dynamics and spectral power shifts in paranoid schizophrenia

Authors: *B. JARRAHI;
Stanford Univ., Palo Alto, CA

Abstract: Paranoid schizophrenia is a subtype of schizophrenia with the predominant features of paranoia, delusions, hallucinations, and other perceptual disturbances. In the present study, we compared the temporal characteristics of large-scale brain networks between schizophrenia patients with and without paranoid features. We leveraged the publicly available schizophrenia

dataset from the Center for Biomedical Research Excellence (COBRE). The dataset included 69 schizophrenia patients (13 females; age range, 18-65 years old) diagnosed based on the Structured Clinical Interview for DSM Disorders. 41 patients in this cohort were classified as paranoid schizophrenia while the remaining patients exhibited other subtypes including residual, disorganized, catatonic, and schizoaffective. In order to investigate the dynamic properties of intrinsic brain activity at rest, we utilized the dynamic Functional Network Connectivity (dFNC) approach derived from group independent component analysis (GICA) of resting state fMRI data. The number of clusters was estimated using Akaike's Information Criterion (AIC), and we computed dFNC using sliding time window correlation and k-means clustering of windowed correlation matrices. Furthering our analysis with k-means, we examined meta-state metrics including the unique windows, state changes, state span, and total travel distance between successive meta-states. Resting state network time series were also processed using Fast Fourier Transform (FFT) to estimate the BOLD spectral power. Differences in dynamic connectivity were found in patients with paranoid schizophrenia in the subcortical, frontoparietal central executive, precuneus, visual, salience, and sensorimotor networks. Notably, patients with paranoid subtype of schizophrenia had higher dFNC between amygdalohippocampal and higher visual networks and lower connectivity between right central executive network and precuneus compared to other subtypes (Bonferroni-corrected $p < 0.05$). In addition to this, between-group differences were observed both in the span of meta-states (i.e., maximum L1 distance between states) and the sum of L1 distances between successive meta-states. The spectral group analysis also showed significant shifts in the power spectra of the subcortical and visual networks in the middle and high frequency bands in paranoid schizophrenia patients. The present study provides novel data about the dynamic aspects of abnormal sensory perception in paranoid schizophrenic patients through investigating temporal dynamics of brain networks.

Disclosures: B. Jarrahi: None.

Poster

PSTR098: Data Analysis and Statistics: Human Brain Structure and Function

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR098.22/Web Only

Topic: J.07. Data Analysis and Statistics

Title: Longitudinal Resting-State fMRI Study Reveals Persistent Alterations in Attention, Default Mode, and Language Networks in Pediatric Post-COVID-19 Subjects.

Authors: *Y. ROJAS-LEMUS[1], S. HIDALGO-TOBON[2], B. DE CELIS ALONSO[3], D. ALVAREZ-AMADO[4], B. ROMERO BAIZABAL[5], J. GARCIA BERISTAIN[4];
[1]Physics, Univ. Autonoma Metropolitana Iztapalapa, Iztapalapa, Mexico; [2]Fisica, UAM-Hospital Infantil de Mexico Federico Gomez, Ciudad DE Mexico, Mexico; [3]Fac. of Physical and Mathematical Sci., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [4]Pediatric Neurol., [5]Imagenology, Hosp. Infantil de Mexico Federico Gomez, Mexico City, Mexico

Abstract: Persistent alterations in large-scale brain networks following SARS-CoV-2 infection in the pediatric population remain poorly characterized. Initial, cross-sectional resting-state functional MRI (rs-fMRI) analysis (2022) of a pediatric post-COVID-19 cohort (n=14; mean age 11.5 [IQR: 10,13]), revealed increased temporal correlations in nodes of the Dorsal Attention Network (DAN) and the Sensorimotor Network [1]. These changes suggested increased intrinsic connectivity in systems associated with visuospatial attention [2] and motor planning [3].The present follow-up study, conducted two years after the initial one, analysed an extended cohort (n=19; mean age 11.7 [IQR: 9, 14]), maintaining identical imaging parameters and preprocessing pipelines (3T scanner; 240 BOLD volumes; TR = 1500 ms; TE = 30 ms; FOV = 250×250 mm^2; voxel size = 2.66×2.66×3.5 mm^3; SMS = 7; scan time = 372 s). The study cohort was contrasted to a paired COVID-free control group (n=31, mean age 10.6 [IQR: 9,11]). Functional data were analyzed using the CONN toolbox [4] with ROI-to-ROI correlation metrics. The study protocol was approved by the Hospital Infantil de México Federico Gómez Ethics Committee and adhered to the principles of the Declaration of Helsinki.The two-year follow-up results showed consistent DAN alterations and additionally revealed altered connectivity in the Default Mode Network (DMN), as well as in Language Networks. These findings suggest SARS-CoV-2 may point to sustained, perhaps maladaptive, neurofunctional reorganization in attentional [2], self-referential, and language systems two years following pediatric SARS-CoV-2 infection. Given established links between DAN dysregulation and attention-deficit/hyperactivity disorder (AD/HD) [5], these results raise concerns about developmental vulnerability. Our results align with post-acute sequelae described in adult COVID-19 survivors [6] and emphasize the neurodevelopmental risks in children undergoing critical periods of brain network maturation. Given the longitudinal nature of the disruptions, our findings advocate for continued neurocognitive monitoring and potential intervention strategies in post-COVID pediatric populations.

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Disclosures: Y. Rojas-Lemus: None. **S. Hidalgo-Tobon:** None. **B. De Celis Alonso:** None. **D. Alvarez-Amado:** None. **B. Romero baizabal:** None. **J. Garcia Beristain:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.01/PP20

Topic: J.08. Methods to Modulate Neural Activity

Support: FWO grant 11Q0X24N
NSTC 113-2640-B-007-001 (Taiwan)

Title: Uncovering the neural effects of transcranial focused ultrasound stimulation: an electrophysiological study in the rat somatosensory cortex

Authors: *C. SMETS, H. HUANG, J.-J. SUN, M. MC LAUGHLIN;
Dept. of Neurosci., KU Leuven, Leuven, Belgium

Abstract: Low-intensity transcranial focused ultrasound stimulation (TUS) is an emerging, non-invasive neuromodulation method with a high spatial resolution and the ability to target deep brain regions. Despite growing interest and promising results in animal and human studies, some challenges remain, including uncertainties around the mechanism and difficulties in reproducing certain effects. To address these concerns, this study investigates the effects of TUS on the somatosensory cortex of anesthetized rats, examining somatosensory evoked potentials (SSEPs), TUS evoked neural activity and neuronal spiking.
Using a 32-channel silicon probe, electrophysiological data was recorded during a 30-min alternating paradigm, in which every other SSEP was stimulated with TUS (500kHz, 1.5 MPa), controlling for the natural variability of SSEPs over time. 14 TUS parameter sets were initially tested, of which 2 were selected for further experiments.
TUS did not significantly alter SSEP metrics across most parameter sets. However, 1 set (0.8 ms PD, 500 Hz PRF, 500 ms PTD) induced significant changes in peak-to-peak values but the effects were inconsistent across animals. Still, this set never induced significant changes in the earbar or visual cortex control. This result suggests that instantaneous effects of TUS are variable, but more research is needed to fully confirm this.
To assess long term effects of TUS, SSEP amplitudes over time were compared between stimulation and control conditions. Although an increase over time was observed with TUS, it was not significantly different from the controls.
It was also investigated if TUS could evoke activity rather than modify ongoing potentials. This analysis showed that TUS (0.2 ms PD, 50 Hz PRF, 500 ms PTD) induced an evoked potential (EP) with a trough at 4 ms and a peak at 6 ms post-stimulation. Similar EPs appeared with visual cortex or earbar stimulation but not in a dead control or during tail stimulation. These findings suggest the EP may be auditory in origin, possibly via bone conduction. However, its persistence after craniotomy challenges this hypothesis. Ongoing studies using deafened rats and auditory stimulation aim to clarify the source.
Lastly, effects of TUS on spiking activity were evaluated. Preliminary results show significant changes in single-unit spiking rates but similar effects occurred in control conditions, requiring further research.
In conclusion, TUS may elicit immediate but variable effects on SSEPs with no significant long-term modulation. Although TUS reliably induces an EP, the origin remains unclear. TUS appeared to influence single-unit spike rates, but this was also observed with the controls.

Disclosures: C. Smets: None. **H. Huang:** None. **J. Sun:** None. **M. Mc Laughlin:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.02/QQ1

Topic: J.08. Methods to Modulate Neural Activity

Title: Enhancing transcranial ultrasound targeting in rats through skull modelling and cone geometry optimization

Authors: *R. PIETERS, C. SMETS, M. MC LAUGHLIN;
KU Leuven, Leuven, Belgium

Abstract: Background: Transcranial focused ultrasound stimulation (TUS) has emerged as a promising non-invasive neuromodulation technique. However, accurate targeting is complicated by skull-induced acoustic distortion, requiring precise calibration and modelling. Commonly, a cone is used in small animals to focus and direct the ultrasound beam. The dimensions of this cone are typically chosen arbitrarily, which may not optimize the pressure reaching the intended target. The cone's geometry must therefore be tailored to the anatomical location of the target to ensure optimal energy transmission.
Objective: This study aims to (1) validate acoustic modelling of rat skull effects against empirical water tank measurements and (2) optimize the design of focusing cones for effective targeting of brain regions at varying depths.
Methods: Three-dimensional acoustic simulations were conducted using k-Wave (MATLAB) and microCT scans from two rat skulls. To assess the accuracy of the modeling, simulated pressure fields were compared with hydrophone measured pressure fields obtained in a in a water tank setup. Subsequently, a *Design of Experiments* approach combined with response surface modeling was used to evaluate and optimize stimulation cone parameters—specifically cone length, tip opening radius, and wall thickness—for maximizing acoustic pressure at targeted brain regions. The medial prefrontal cortex (mPFC) and cerebellum were selected as targets for this study, illustrating how optimal cone geometry can vary depending on the anatomical target.
Results: Modelled pressure fields showed high agreement with experimental measurements. Skull attenuation and refraction effects were successfully accounted for in the model. Optimized cone designs significantly improved energy delivery to both mPFC and cerebellum targets.
Conclusion: Validated acoustic modelling enables accurate prediction of ultrasound transmission through the rat skull, making it a valuable tool for TUS planning. Tailored cone designs enhance target-specific delivery, supporting precise neuromodulation studies in small animals. These results lay groundwork for reproducible TUS applications in preclinical neuroscience.

Disclosures: R. Pieters: None. **C. Smets:** None. **M. Mc Laughlin:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.03/QQ2

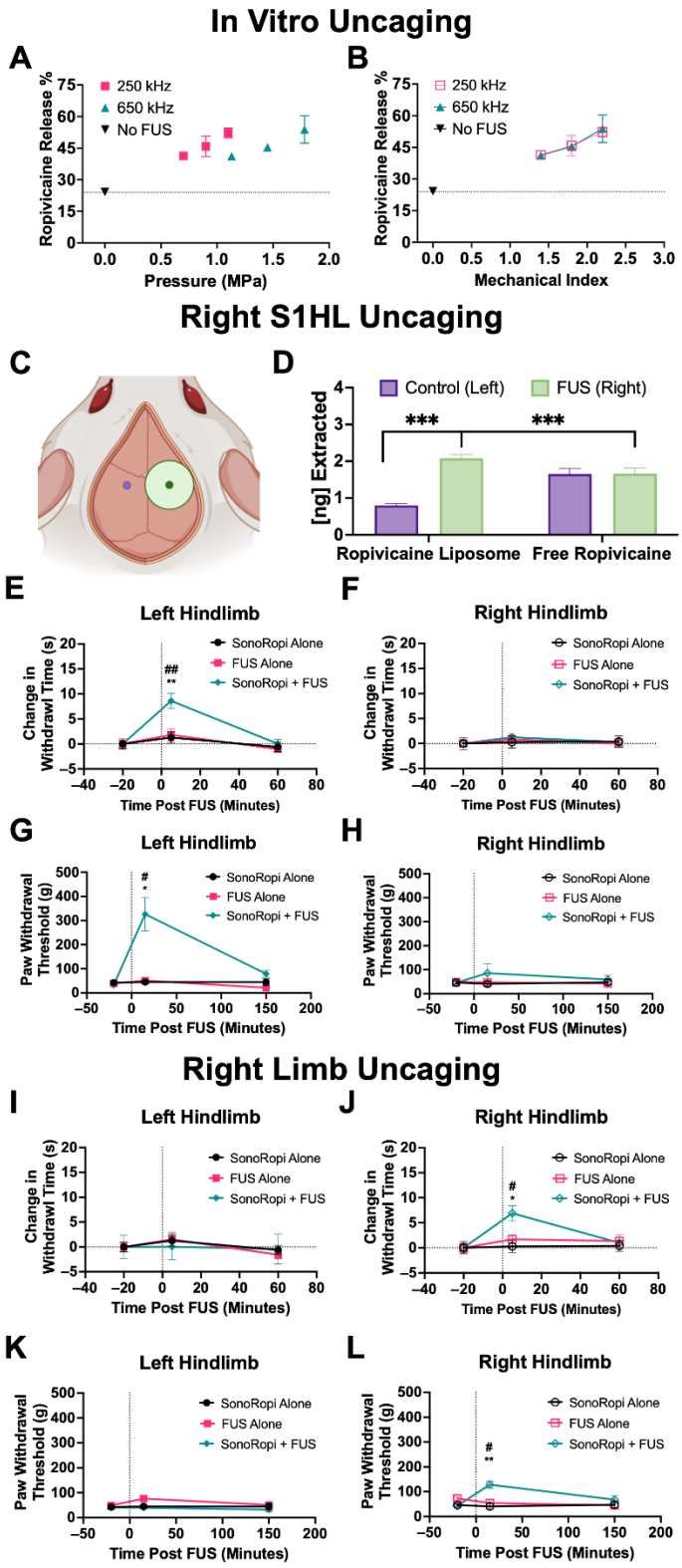
Topic: J.08. Methods to Modulate Neural Activity

Support: NIH/NINDS UG3NS115637

Title: Targeted reversible brain and limb anesthesia via focused ultrasound-assisted liposomal ropivacaine uncaging

Authors: *P. MARTINEZ[1], Y. XIANG[4], K. SINHA ROY[1], M. PUROHIT[5], B. YU[2], R. AIRAN[3];
[1]Stanford Univ., Stanford, CA; [2]Stanford Univ., Palo Alto, CA; [3]Radiology, Stanford Univ., Stanford, CA; [4]Stanford Med. Dept. of Radiology, Stanford, CA; [5]Stanford, Stanford, CA

Abstract: Ropivacaine is a sodium channel blocker that crosses both the blood-nerve barrier and blood-brain barrier indicating it may be used for both peripheral and central regional anesthesia [1,2] though it poses cardiovascular and central nervous toxicity risks [3,4]. To enable ropivacaine delivery to target brain regions or peripheral nerves, we encapsulated it into acoustically-activatable liposomes (SonoRopi), allowing release only at focused ultrasound (FUS)-targeted sites.Liposomes were prepared via extrusion and loaded using a transmembrane ammonium sulfate gradient. In vitro release was tested using 250 and 650 kHz transducers. For in vivo studies, rats were infused with SonoRopi (2 mg/kg) and sonicated with 250 kHz FUS (1.1 MPa) targeting the right somatosensory cortex. Solid phase microextraction (SPME) probes measured local drug accumulation, and behavioral effects were assessed via hot plate and von Frey tests before and after treatment.SonoRopi showed pressure-dependent release in vitro, with release scaling with mechanical indices across frequencies (A, B). In vivo, FUS targeting led to higher ropivacaine accumulation in the right S1HL (2.08 ng) vs. control (0.8 ng, F). Free drug distributed evenly between hemispheres (1.65 ng each, F). Rats receiving SonoRopi + S1HL FUS exhibited significantly increased paw withdrawal latency (8.6 s) and higher mechanical thresholds (327.8 g) in the contralateral hindlimb, with no significant changes in the ipsilateral limb or in control groups (D-H). Effects subsided by 60 minutes post-treatment. Limb FUS + SonoRopi showed significantly increased paw withdrawal times (6.9s) and increased von Frey thresholds (128.3 g), both on the treated limb. All other groups showed no significant differences, all effects reduced after 2.5 hours (I-L). Our study shows that combining SonoRopi with focused ultrasound effectively modulates brain and peripheral nerve activity in a region specific manner. This innovative approach offers significant potential for functional brain mapping and acute and chronic pain therapies.


In Vitro Uncaging
A
Ropivicaine Release %
250 kHz
650 kHz
No FUS
Pressure (MPa)
B
Mechanical Index
Right S1HL Uncaging
C
D
Control (Left)
FUS (Right)
[ng] Extracted
***
***
Ropivicaine Liposome
Free Ropivicaine
E
Left Hindlimb
F
Right Hindlimb
SonoRopi Alone
FUS Alone
SonoRopi + FUS
Change in Withdrawl Time (s)
Time Post FUS (Minutes)
G
Left Hindlimb
H
Right Hindlimb
Paw Withdrawal Threshold (g)
Right Limb Uncaging
I
Left Hindlimb
J
Right Hindlimb
K
Left Hindlimb
L
Right Hindlimb

Disclosures: P. Martinez: None. **Y. Xiang:** None. **K. Sinha Roy:** None. **M. Purohit:** None. **B. Yu:** None. **R. Airan:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.04/QQ3

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS124564
NIH Grant RF1NS131069
NIH Grant R01NS127849
NIH Grant R01NS096761
NIH Grant T32EB029365
NSF Grant DGE2140739

Title: Transcranial focused ultrasound induces source localizable cortical activation in resting state humans when applied concurrently with transcranial electric stimulation

Authors: *J. KOSNOFF[1], C. GONSISKO[2], K. YU[1], J. ZHANG[2], Y. DING[2], Y. ZHANG[2], B. HE[3];
[1]Biomed. Engin., [3]Dept. of Biomed. Engin., [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The effects of transcranial focused ultrasound (tFUS) on the human brain are poorly understood. Currently, the field is at odds with whether tFUS in humans is subthreshold modulating the brain's excitability towards other stimuli, producing suprathreshold neural stimulation on its own, or if it even has a spatially specific non-auditory induced affect. We investigated the ability of excitatory (e) and inhibitory (i) tFUS, transcranial direct current stimulation (tDCS) and a novel combination of the two (transcranial electro-acoustic stimulation; tEAS) to evoke cortical target-location specific activity in 22 resting state humans with high density electroencephalography (EEG) recordings. etFUS with 3kHz pulse repetition frequency to left motor cortex induced 30, 90, and 170 ms exogenous potentials, which presented with hemisphere-symmetric responses. In 21 of the 22 subjects with etFUS, we also tested a parameter-matched right prefrontal cortex spatial control condition. Bayes Factor analysis of the left-hemisphere electrodes at region of interest (ROI) for the two conditions provided robust evidence that the resting state brain responses of tFUS targeted to two distinct brain regions were statistically similar ($\log_{10}$ BF < 0 for all ROI electrodes). EEG data were back projected to the subjects' cortices using the dynamic statistical parametric mapping (dSPM) method, and the resultant power of source waveforms were averaged over the 10 - 63 ms window (identified from sensor-level permutation cluster tests). Source-morphing to a common brain model and averaging the response across subjects showed strong concordance with the targeted ROI. Statistical analysis to determine if ipsilateral (IL) ROI power was significantly higher than the homologous contralateral (CL) power was conducted with permutation t-tests with false-

discovery rate multiple comparison correction. Both tEAS conditions had significantly increased IL ROI source power (etEAS IL = 2.61 ± 1.21 z_{dSPM}, CL = 1.66 ± 0.66 z_{dSPM}, $p_{adj} < 0.01$; itEAS IL =2.39 ± 1.14 z_{dSPM}, CL = 1.20 ± 0.47 z_{dSPM}, $p_{adj} < 0.01$). None of the other conditions had significantly increased IL source power (all $p_{adj} > 0.05$). Out of all conditions tested, only the co-modulatory combination of tEAS yielded source localizable signals. This is the first human study, to our knowledge, to harness and demonstrate the electro-acoustic compounding effect in neural stimulation. In doing so, we developed a new non-invasive technique that does not just modulate, but rather stimulates, targeted brain regions. Perhaps even more critically, the data present much-needed evidence that tFUS is a subthreshold co-modulator of the human cortex.

Disclosures: J. Kosnoff: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent application for tFUS technique. **C. Gonsisko:** None. **K. Yu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent for tFUS technique. **J. Zhang:** None. **Y. Ding:** None. **Y. Zhang:** None. **B. He:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pending patent application for tFUS technique.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.05/QQ4

Topic: J.08. Methods to Modulate Neural Activity

Support: JP24K02357
JP24gm6510013

Title: Ultrasound-induced neuromodulation of cortical neurons through trpc6 channel activation

Authors: *M. SHIMOJO[1], Y. MATSUSHITA[2], Y. TAKEUCHI[3], M. HIGUCHI[1];
[2]Advanced Neuroimaging Ctr., [1]Natl. Inst. for Quantum Sci. and Technol., Chiba, Japan; [3]Kindai Univ., Osaka, Japan

Abstract: Neuromodulation is a fundamental biomedical approach that directly alters nerve activity in brain circuits through various external stimuli. Among the diverse modalities available, ultrasound has recently received significant attention as a promising tool for neuromodulation due to its non-invasiveness, excellent tissue permeabilization, and high spatiotemporal precision. Despite its growing utility in neuroscience research and clinical applications, the detailed mechanisms by which ultrasound influences brain circuits remain largely unknown. To address this critical gap, we employed experimental configurations that allow side-by-side assessment of neuronal activity elicited by ultrasound in both cortical neuronal cultures and the living mouse brain. We investigated the molecular and cellular

mechanisms underlying ultrasound neuromodulation in mammalian neurons. First, we established a live-cell imaging platform based on fluorescence microscopy, integrated with an ultrasonic transducer, to examine how ultrasound stimulation affects Ca^{2+} oscillations in mammalian neurons. Using this setup, we demonstrated that pulsed ultrasound irradiation induces rapid Ca^{2+} transients in cultured neurons. This neuronal response was found to depend on extracellular Ca^{2+} influx, action potential generation, and synaptic transmission. Notably, both pharmacological inhibition and genetic disruption of TRPC6 nearly abolished the neuronal response to ultrasound. Furthermore, in vivo electrophysiological analysis consistently confirmed the essential role of TRPC6 in transcranial ultrasound neuromodulation. Taken together, our findings suggest a sequential mechanism in which TRPC6 activation, triggered by acoustic pressure, constitutes a major pathway in ultrasound neuromodulation. This study provides fundamental insights into the molecular and cellular basis of ultrasound-mediated neuronal regulation, supporting the future development of safe and finely tunable ultrasound-based neuromodulation strategies.

Disclosures: M. Shimojo: None. **Y. Matsushita:** None. **Y. Takeuchi:** None. **M. Higuchi:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.06/QQ5

Topic: J.08. Methods to Modulate Neural Activity

Support: KIST Institutional Program 2E33771

Title: Evaluation of auditory confounds in transcranial focused ultrasound brain stimulation using cortical recording

Authors: *J. KUM[1,2,3], S. LEE[4], S. HYUN[1,5], D. SUNG[1], W. LEE[1,2], H. KIM[1,2,5];
[1]Bionics Res. Ctr., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Div. of Bio-Medical Sci. & Technol., Univ. of Sci. and Technology, KIST Sch., Seoul, Korea, Republic of; [3]Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon, Korea, Republic of; [4]Dept. of Electrical and Computer Engineering, Sungkyunkwan Univ., Suwon, Korea, Republic of; [5]KHU-KIST Dept. of Converging Sci. and Technology, Kyunghee Univ., Seoul, Korea, Republic of

Abstract: In the past decade, transcranial focused ultrasound (tFUS) has emerged as a promising non-invasive brain stimulation technique, offering bimodal neuromodulation (i.e., excitation and inhibition) with exquisite spatial resolution and deep tissue penetration. In 2018, it was reported that tFUS-mediated neuromodulation may be accompanied by unintended auditory confounding effects, raising concerns about attributing observed response solely to the stimulation of targeted brain regions. Therefore, decoupling auditory confounds from tFUS-induced brain responses is

crucial for accurately analyzing the effects of ultrasound neuromodulation. In this study, we aimed to perform electrophysiological assessments of auditory confounds associated with tFUS stimulation targeting non-auditory cortices. We recorded cortical responses via electroencephalography (EEG) or electrocorticography (ECoG) in urethane-anesthetized male Sprague-Dawley rats. The left primary auditory cortex (A1), along with bilateral motor, somatosensory, and visual cortices, was monitored during tFUS sessions. A custom-built 300-kHz miniature transducer was positioned over the right primary visual cortex. 0.2 s tFUS stimulations, pulsated with a 0.1 or 1 kHz pulse repetition frequency, were administered with a 5 s pulse train repetition interval at an acoustic pressure of 211 kPa or a spatial-peak temporal average intensity (I_{SPTA}) of 750 mW/cm^2 to be matched across different experimental conditions. In selected experiments, we also applied customized envelopes to the tFUS actuation signals to investigate the potential for reducing auditory responses. Cortical responses during tFUS stimulation were observed across all recorded regions, with characteristics suggestive of auditory confounding effects. When envelope shaping was applied, the amplitude of event-related potentials decreased, and normalized power in the delta to low-gamma frequency range (1-50 Hz) at A1 was attenuated up to 56%. This effect persisted even when the acoustic pressure was increased to match the I_{SPTA} of conventional pulsed stimulation. Responses in other cortices, which exhibited temporal and spectral relationships with A1 activity, were also reduced when A1 responses were diminished through envelope shaping. Our findings demonstrate that the tFUS-induced auditory confounds are highly dependent on the envelope shape of the pulsed stimulation, rather than other sonication parameters. This study highlights the importance of waveform design in isolating the direct neuromodulatory effect of tFUS—an essential step towards its precise application in both research and clinical contexts.

Disclosures: J. Kum: None. **S. Lee:** None. **S. Hyun:** None. **D. Sung:** None. **W. Lee:** None. **H. Kim:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.07/QQ6

Topic: J.08. Methods to Modulate Neural Activity

Title: Development of a Novel Ultrasound Adapter for Targeted Brain Stimulation

Authors: Y. MURAI[1], ***F. YOSHIDA**[2];
[1]Physiol., Dept. of Physiology, Kurume Univ. Sch. of Med., Kurume/Fukuoka, Japan; [2]Kurume Univ., Kurume-Shi, Japan

Abstract: Ultrasound-based brain stimulation has emerged as a promising non-invasive technique for modulating neural activity, with growing interest in its potential therapeutic applications in neuroscience. Previous studies have explored the use of ultrasound to stimulate specific brain regions, aiming to enhance brain function. However, traditional methods often rely

on degassed water, which not only introduces considerable preparation time but also suffers from low reliability, thus limiting its practical application. To overcome these challenges, the present study introduces a novel adapter that eliminates the need for degassed water, offering a more efficient and reliable approach to ultrasound stimulation. This adapter allows for rapid attachment and ease of use, making it accessible for routine experimental procedures.The primary objective of this study was to evaluate the effectiveness of the newly developed adapter in addressing the shortcomings of conventional methods, specifically focusing on reducing preparation time and improving the success rate of ultrasound stimulation. The adapter is designed to be easily applied with a single touch, ensuring that the method is simple and accessible for users of various skill levels. Additionally, the adapter's tip, with a diameter of 6 mm, is specifically designed to enable non-invasive stimulation of deep brain regions, which is critical for accurately targeting and modulating specific neural structures. This innovation is expected to significantly streamline experimental protocols and enhance the reproducibility of results. In this study, a commercial ultrasound generator was utilized, and a custom adapter was designed in collaboration with a company. To assess the functional impact of the stimulation, the motor cortex was targeted, and the induction of upper limb motor responses was monitored. Electromyography (EMG) was recorded needle electrodes, allowing for precise monitoring of motor responses. The results confirmed that no thermal effects were observed, and ultrasound stimulation successfully induced motor activity in the upper limbs.The findings from this study demonstrate that the newly developed adapter significantly reduces preparation time while maintaining high safety and reliability, making it a highly valuable tool for non-invasive brain stimulation. The successful development and validation of this adapter represent an advancement in the field of ultrasound-based brain stimulation and offer a practical solution for research applications.

Disclosures: Y. Murai: None. **F. Yoshida:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.08/QQ7

Topic: J.08. Methods to Modulate Neural Activity

Title: Transcranial Focused Ultrasound Alters Neuronal Excitability and Spike Shape in a Parameter-Specific Manner in Rats

Authors: *H. HUANG[1], C. SMETS[2], L. CHEN[3], M. MC LAUGHLIN[1];
[1]Neurosci., KU Leuven, Leuven, Belgium; [2]Neurosci., Dept. of Neuroscience, KU Leuven, Leuven, Belgium; [3]Neurosci., KU LEUVEN, LEUVEN, Belgium

Abstract: Background: Transcranial focused ultrasound stimulation (TUS) is a novel non-invasive neuromodulation technique offering precise spatial targeting and deep penetration. It operates via acoustic pressure waves that potentially influence neuronal membrane potentials,

ion channels, and synaptic transmission, yet its precise mechanisms remain poorly understood. In animal models, TUS has demonstrated both excitatory and inhibitory effects, highlighting the need for systematic parameter optimization. This study aims to evaluate TUS's impact on motor cortex electrophysiological activity in rats with different parameters, providing insights into its potential as a neuromodulatory tool. **Methods**: 15 adult SD rats received TUS targeting the motor cortex. Specifically, we investigated how varying pulse repetition frequency (PRF; 25-2000 Hz) and pulse duration (PD; 50-800 μs) affected neuronal firing patterns and spike waveform characteristics, which includes spike width (duration between initial and final deflection points), peak amplitude (maximum positive amplitude), trough amplitude (maximum negative amplitude following the peak), and repolarization time (time taken from the peak amplitude back to baseline). Other stimulation parameters were fixed at 500 kHz fundamental frequency, 1-second inter-sonication interval, and ~1 MPa pressure. Neural recordings were collected using a 32-channel silicon probe. Each session comprised before, during and after-stimulation periods, each lasting 3 minutes. Data preprocessing was done with MATLAB, and spike sorting performed using Kilosort 4.0. **Results**: Analysis across 15 rats using linear mixed effects models revealed significant, parameter-dependent modulation of spike rates and spike waveform dynamics. Notably, PRF at 500 Hz elicited the strongest spike rate increase ($p<0.0001$), while significant waveform alterations, including decreases in spike width, were consistently observed at tested PRFs (25, 50, 500 and 2000 Hz). Repolarization time and trough amplitudes, however, showed minimal modulation by PRF. Additionally, we observed significant effects of pulse duration on spike rate, particularly at 50 μs ($p<0.0001$). However, no significant changes were observed in peak, trough, and repolarization times. **Conclusion**: This preliminary study suggests that TUS has potential for modulating motor cortex neuronal activity in a parameter-dependent manner, highlighting the importance of optimizing stimulation parameters to achieve reliable and targeted neuromodulatory outcomes. Further research is needed to understand TUS's mechanisms and ensure consistent outcomes.

Disclosures: H. Huang: None. **C. Smets:** None. **L. Chen:** None. **M. Mc Laughlin:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.09/QQ8

Topic: J.08. Methods to Modulate Neural Activity

Title: Temporal Patterning of Low-Intensity Focused Ultrasound Influences the Degree of Neuromodulation in the Human Motor Cortex

Authors: *G. ISAAC[1], W. LEGON[2];
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Abstract: Temporal patterning has been recognized as a key parameter influencing outcomes in other brain stimulation modalities, including deep brain stimulation (DBS) and transcranial

magnetic stimulation (TMS), but its role in Low-Intensity Focused Ultrasound (LIFU) remains largely unexplored. Here, we investigated how seven distinct LIFU temporal patterns, compared to an active sham, modulate corticospinal excitability in healthy human participants. Using a finger-tapping fMRI task, we first localized each subject's first dorsal interosseous (FDI) representation in the primary motor cortex (M1). In subsequent sessions, participants received 10 seconds of 500 kHz LIFU precisely targeted to peak fMRI activation within the M1 using stereotactic neuronavigation. Following each stimulation pattern, corticospinal excitability was assessed using motor evoked potentials (MEPs) elicited by single-pulse TMS. We applied strict go/no-go criteria to minimize the potential effect of carry-over between conditions and used auditory masking to reduce non-specific auditory effects. Analysis of preliminary data (N = 4) revealed distinct effects of temporal patterning on (normalized) post-LIFU MEP amplitudes (mean ± SEM): Pattern 1 (0.99 ± 0.23), Pattern 2 (0.76 ± 0.15), Pattern 3 (0.88 ± 0.14), Pattern 4 (0.71 ± 0.13), Pattern 5 (0.82 ± 0.07), Pattern 6 (0.63 ± 0.13), Pattern 7 (0.88 ± 0.20), and Sham (1.24 ± 0.35). Patterns 4 and 6 showed the greatest reductions in MEP amplitude. These early findings suggest that temporal patterning is a potent, underutilized dimension for optimizing LIFU neuromodulation. Ongoing data collection will assess reproducibility and inform protocol design for future therapeutic applications.

Disclosures: G. Isaac: None. **W. Legon:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.10/QQ9

Topic: J.08. Methods to Modulate Neural Activity

Title: Transcranial ultrasound stimulation on human somatosensory cortex disrupts motor memory consolidation

Authors: *S. ABBASIKAMAZANI, L. HOU, Y. LEI;
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Abstract: Human newly learned movements are labile and undergo a consolidation process to form the long-term motor memory. Force-field adaptation learning is associated with changes to the representation of limb position in the somatosensory cortex. This study aimed to test if the inhibition of somatosensory cortex activity after learning could disrupt memory consolidation and block memory retention. Thirty participants made reaching movements while gradually being exposed to a robotic dynamic force field. Immediately after the force adaptation training, a 500 kHz four-element annular array ultrasound transducer with the personalized focal depth was utilized to stimulate the somatosensory cortex of 15 participants. The sonication paradigm was an 80-second pulse train with pulse repetition frequency of 5 Hz and duty cycle of 10%. The estimated intracranial spatial-peak pulse-average intensity (I_{SPPA}) was 2.38 W/cm^2. Another 15 participants were assigned to the sham condition with no ultrasound stimulation. Following a 6-

hour delay, all participants were tested for memory retention and relearned the force adaptation task. Our results showed that the inhibition of somatosensory cortex following learning impaired memory retention, compared with the sham condition. These findings are consistent with the idea that the somatosensory cortex is part of a circuit that is involved in motor memory consolidation.

Disclosures: S. Abbasikamazani: None. **L. Hou:** None. **Y. Lei:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.11/QQ10

Topic: J.08. Methods to Modulate Neural Activity

Title: Effects of transcranial ultrasonic stimulation on cortical activity and pain affect in rodents

Authors: *E. HU[1], Q. ZHANG[2], Y. ZHU[3], T. LEMAIRE[4], S. SHOHAM[5], J. WANG[6]; [1]New York Univ. Grossman Sch. of Med., New York, NY; [2]Anesthesiol., NYU Sch. of Med., New York, NY; [3]Stanford Univ., Stanford, CA; [4]New York Univ., Long Island City, NY; [6]Anesthesiol., [5]NYU Grossman Sch. of Med., New York, NY

Abstract: Chronic pain is a costly and pervasive clinical challenge, with few reliable long-term treatments burdened by significant side effect risk. Recently, transcranial ultrasonic stimulation (TUS) has emerged as an enabling technology for the development of a central pain therapeutic, owing to its excellent spatial targetability and rapid titratability when compared to other noninvasive neuromodulatory methods. Furthermore, it is known that the anterior cingulate cortex (ACC) provides both lateral and top-down regulation of affective pain perception, making it an excellent target for the development of a TUS-based analgesic. However, while TUS encompasses a broad space of possible stimuli defined by a range of waveform parameters (e.g. pulse pressure, duty cycle, repetition frequency), the effects of these parameters on neuronal activity and behavior remain poorly understood. In the first part of the study, we aimed to establish the potential efficacy of TUS in reducing pain-related behavior through conditioned place aversion (CPA) assays in a preliminary cohort of two wild-type rats. As expected, we found that subjects tend to avoid the chamber associated with acute mechanical pinprick. However, this effect appeared to reverse in chambers associated with both pinprick and low intensity TUS sonication, supporting the pain-ameliorating effect of TUS. Next, we conducted acute *in vivo* electrophysiological recordings within a wild-type rat ACC using a high-density silicon probe, while delivering focal TUS sonication and varying excitation waveforms across four points across the parameter space. Additionally, we designed and implemented stimulation protocols optimized for minimizing electromechanical artifacts. We identified a consistent and delayed increase in power across all local field potential frequency bands following the sonication offset (200 ms), followed by a quiescent period and a secondary peak (500 ms). Together, these results support the use of the ACC as an effective superficial target for

ultrasound-based pain treatment, while revealing the complexity of cellular responses to ultrasonic stimulation.

Disclosures: E. Hu: None. **Q. Zhang:** None. **Y. Zhu:** None. **T. Lemaire:** None. **S. Shoham:** None. **J. Wang:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.12/QQ11

Topic: J.08. Methods to Modulate Neural Activity

Title: Targeted Sonogenetic Neuromodulation of Piezo1 Using Focused Ultrasound

Authors: *Y. LEE[1], S. KOO[2], M. KWAK[3], J. CHEON[4];
[1]Yonsei Univ. IBS CNM, Seoul, Korea, Republic of; [2]IBS CNM, [4]IBS Ctr. for Nanomedicine, [3]Yonsei Univ., Seoul, Korea, Republic of

Abstract: Precise control over neuronal activity is fundamental to comprehending brain function and developing targeted therapeutic interventions. Sonogenetics is a minimally invasive neuromodulation technology that utilizes focused ultrasound (FUS) in combination with ion channels to remotely activate genetically modified neurons for brain stimulation. Among these, Piezo1, a calcium-permeable mechanosensitive channel, offers a compelling candidate due to its robust mechanical responsiveness. However, the underlying mechanisms by which focused ultrasound excites neurons remain unclear, posing substantial challenges for the application of this technology in research and potential clinical settings for neurodegenerative disorders. Here, we engineered a custom-designed helmet ultrasound system for freely moving mice. Overexpressing Piezo1 in neurons *in vivo* enables controlled ultrasound activation and downstream functional readouts and a stronger response in neurons using focused ultrasound. Here, we observed induced upregulation of GFAP, Iba1, and c-Fos as biological markers of successful intracranial energy deposition at the M2 motor cortex target region. This platform allows for precise delivery of mechanical energy into the brain and provides insight into the biological consequences of Piezo1 activation, with potential applications in both basic neuroscience and therapeutic neuromodulation.

Disclosures: Y. Lee: None. **S. Koo:** None. **M. Kwak:** None. **J. Cheon:** None.

Poster

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Topic: J.08. Methods to Modulate Neural Activity

Support: French national research agency (ANR) under the references ANR-20-CE37-0005
Exploratory Program of CEA, the French Alternative Energies and Atomic Energy Commission

Title: Focused ultrasound-induced BBB opening enables noninvasive, site-specific oxytocin delivery in primate brain, modulating amygdala activity and connectivity

Authors: E. DJABALLAH[1], C. CORNU[2,3], G. PAGÉ[3], B. LARRAT[2,3], *Q. ZHU[1];
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Abstract: Social dysfunction is a hallmark of psychiatric and neurodevelopmental disorders, yet effective treatments remain limited. Intranasal oxytocin (OT) shows promise but is still hindered by poor blood-brain barrier (BBB) penetration (Lee et al., 2018, 2020; Gulliver et al., 2019; Yamasue et al., 2018), restricting research and clinical utility. Site-specific OT administration in primates—particularly humans—is critical for studying social cognition circuits, but also lacking non-invasive tools. Here, we developed a novel approach to deliver OT directly to specific brain regions using focused ultrasound (FUS)-induced BBB opening in primates, validated via 7T pharmacological functional MRI (phMRI). Using a clinical-grade FUS system with real-time microbubble cavitation monitoring (Kamimura et al., 2019; Mondou et al., 2025), we targeted the central amygdala (CeA) in four anesthetized rhesus macaques (2 left, 2 right hemispheres, 2 males). BBB opening was guided by neuronavigation and confirmed via contrast-enhanced T1-weighted MRI (GRE, 0.8 mm isotropic; Dotarem). Post-opening, phMRI (2D SMS EPI, 1 mm isotropic, 1.96 s TR) assessed OT brain penetration. Saline and OT (25 IU, 6-10 mL, 150 mL/h) were IV-infused during separate 40-minute scans (OT first), preceded by baseline periods. Control sessions (no BBB opening) with identical infusions were conducted ≥2 weeks later, forming a 2×2 design (BBB status ×infusion type). In all subjects, FUS successfully opened the CeA BBB; no tissue damage was observed via MRI, even after repeated sessions. GLM analysis revealed OT-induced sustained activation in the CeA only during BBB-opened sessions (vs. saline), supported by a significant interaction between BBB status and infusion type. Interaction effects also occurred in social network regions: Nucleus Basalis of Meynert, putamen, and orbital frontal cortex. Seed-based connectivity analysis (CeA as seed) showed increased functional connectivity with the Nucleus Basalis, putamen, and midbrain dopaminergic complex exclusively when OT was delivered post-BBB opening. These findings demonstrate for the first time that FUS-induced BBB opening enables non-invasive, site-specific OT delivery to primate brains, confirming OT penetration into the CeA and its modulation of social network circuits. This method holds substantial promise to advance primate and human oxytocin research, potentially enabling more effective treatments for social dysfunction.

Disclosures: E. Djaballah: None. **C. Cornu:** None. **G. Pagé:** None. **B. Larrat:** None. **Q. Zhu:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

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Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.14/QQ13

Topic: J.08. Methods to Modulate Neural Activity

Title: Understanding the causal role of the insula to predictable and unpredictable threat: an fMRI study

Authors: *S. GHOLIPOUR PICHA[1,2], W. LEGON[2];
[1]Virginia Tech., Roanoke, VA; [2]Fralin Biomed. Res. Inst., Roanoke, VA

Abstract: Background:
Heightened reactivity to both predictable and unpredictable threat is a hallmark of anxiety, yet the causal roles of salience network nodes in shaping these responses remain poorly understood. The dorsal anterior insula (dAI) and anterior mid-cingulate cortex (aMCC) are key hubs implicated in the integration of interoceptive, emotional, and autonomic information, but prior evidence has been largely correlational due to limited access to these deep targets. Here, we used low-intensity focused ultrasound (LIFU), a non-invasive neuromodulation method with deep millimeter-scale precision, to transiently modulate activity in either the dAI or aMCC in healthy participants performing the No-Predictable-Unpredictable (NPU) threat task.
Methods:
Participants performed a No, Predictable, Unpredictable (NPU) threat task inside a 3 T MAGNETOM Prisma. Two blocks of the NPU task were run (PNUNUNPNPNUNUNP & UNPNPNUNUNPNPNU). Within each block each letter trial lasted 13 s (5 s Cue, 8 s no-Cue). 24 total electric shocks with short duration (2 ms) were delivered titrated to an 7/10 unpleasant intensity to participants' non-dominant forearm. Functional images were acquired at TR = 1.5 s, TE = 34 ms, 2 mm isotropic voxels (96×96×72), plus T1w (0.9 mm isotropic), resting state, and Diffusion Kurtosis Imaging (DKI). Preprocessing was performed with our own custom pipeline incorporating FSL, AFNI, and ANTs packages commands. The insula was defined a priori as ROI, with primary contrasts U > N and P > N. LIFU will target insular subregions showing anxiety-related hyperactivity or dysconnectivity and its parameters will be individualized based on fMRI/functional-structural connectivity results.
Results:
Preliminary data from fMRI analysis confirm robust insular activation to Unpredictable and Predictable vs. No shock. For some participants, regarding significant activation, for P > N, left dorsal agranular insula (LDAI) is spatially more than 60% activated, while left dorsal dysgranular insula (LDDI) is more than 30% activated; also, for U > N, LDAI is more than 40% activated. For some other participants, for P > N, LDDI and RDAI are more than 50% activated while RDDI and LDAI are more than 40% activated; also, for U > N, RDAI is more than 60% activated while LDAI is more than 50% activated.
Conclusions:
These early findings demonstrate strong involvement of the insula in the anticipation of threat.

Targeting these subject specific regions within the insula with LIFU may serve as a means to reduce activity to reduce response to threat demonstrating LIFU's promise for probing, and potentially treating, deep-brain circuit dysfunction in anxiety-related disorders.

Disclosures: S. Gholipour Picha: None. **W. Legon:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

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Program #/Poster #: PSTR099.15/QQ14

Topic: J.08. Methods to Modulate Neural Activity

Title: Investigating axonal responses to ultrasound neuromodulation in vivo

Authors: *E. VICARI[1], T. LEMAIRE[2], T. TARNAUD[3], O. RIZZO[4], T. PLOVIE[3], O. AKOUISSI[1], B. RODRÍGUEZ-MEANA[5], N. D. JAMES[1], G. COURTINE[1], E. TANGHE[3], E. NEUFELD[6], S. MICERA[1,5];
[1]EPFL, Genève, Switzerland; [2]New York Univ., Long Island City, NY; [3]Ghent Univ., Ghent, Belgium; [4]Univ. of Geneva, Geneva, Switzerland; [5]SSSA, Pisa, Italy; [6]Fndn. for Res. on Information Technologies in Society, Zurich, Switzerland

Abstract: Focused ultrasound is increasingly adopted for the non-invasive neuromodulation of brain circuits. However, its impact on axons - crucial for applications involving peripheral neuropathies and long-range brain connectivity - remains poorly characterized, as current evidence of direct effects is limited to *ex vivo* studies. Hence, it is unclear whether focused ultrasound can reliably modulate axonal firing rates in physiologically relevant *in vivo* models. In this study, we investigated the direct excitatory and modulatory effects of focused ultrasound on spinal roots in anesthetized rats. A laminectomy was performed to access the lumbar spinal cord, and stimulation was applied to the exposed isolated roots. Neural and muscular responses in the hindlimb were used as indicators of effective neuromodulation. Information on modulation was primarily derived from measurements of firing rate, shape and latency of compound action potentials, and amplitude of evoked contractions in antagonist muscles. Electrical stimulation served as a reference modality to interpret the ultrasound-evoked responses. In parallel, finite element simulations were conducted to estimate mechanical and thermal axon exposure conditions during sonication. Candidate biophysical mechanisms of ultrasound-induced cellular effects were integrated into neural axonal models that were simulated under the same exposure conditions to correlate mechanistic predictions with the experimentally observed responses. Our findings demonstrate that, within an identified parameter space, ultrasound can reliably elicit neural activity in the sciatic nerve and evoke muscle contractions in hindlimb antagonist muscles. Ultrasound-evoked responses closely resembled those obtained with electrical stimulation and muscle activation patterns were confirmed to be specific to the targeted spinal root. Notably, the modulation of firing rate was successfully controlled through variation of ultrasound parameters, and the recovery dynamics following ultrasound-evoked action potentials

enabled neuromodulation at physiologically relevant rates. The comparison between simulated mechanical and thermal exposure conditions, mechanistically predicted neural activity, and experimentally recorded responses provides insights into the underlying biophysical mechanisms of axonal activation through ultrasound. These findings advance our understanding of the neuromodulatory potential of this technique, permit targeted optimization of future experimental designs and stimulation protocols, and inform future translational applications.

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Poster

PSTR099: Transcranial Ultrasound Stimulation

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Program #/Poster #: PSTR099.16/QQ15

Topic: J.08. Methods to Modulate Neural Activity

Support: University of Minnesota's MnDRIVE (Minnesota's Discovery, Research and Innovation Economy) initiative

Title: Preliminary validation of target engagement in low intensity focused ultrasound modulation in human motor cortex

Authors: *S. ADAMS[1], A. S. WIDGE[2];
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Abstract: Low-intensity focused ultrasound (LIFU) holds significant potential as a non-invasive neuromodulation method capable of targeting deep brain structures inaccessible to conventional transcranial magnetic stimulation (TMS). However, reliable biomarkers of LIFU-induced neural modulation are required to validate clinical utility. This preliminary study aimed to identify reproducible markers of LIFU engagement by assessing modulation of the primary motor cortex (M1) in healthy human participants.We delivered LIFU using a Brainsonix neuromodulation device at parameter sets previously established to induce cortical excitability. Cortical responses were quantified via motor-evoked potentials (MEPs) elicited by single-pulse and paired-pulse TMS, recorded from the first dorsal interosseous muscle through electromyography (EMG). A randomized block design with active and sham sonications assessed response reproducibility and specificity.Preliminary results demonstrate significant increases in MEP amplitudes under specific active LIFU conditions compared to sham, confirming neuromodulatory effects. These data provide initial validation for MEP-based biomarkers as measures of LIFU target engagement and offer critical insights into optimal stimulation parameters. Ongoing analyses are focused on refining dose-response characteristics and temporal dynamics, contributing foundational knowledge for LIFU's clinical translation in neurological and psychiatric disorders.

Disclosures: S. Adams: None. **A.S. Widge:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

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Topic: J.08. Methods to Modulate Neural Activity

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Title: Transparent ultrasound cranial window for simultaneous ultrasound neuromodulation and optical imaging in awake mice

Authors: *S. MIRG[1], K. SAMANTA[3], P. GADDALE[4], F. SALEHI SHAHRBABAKI[6], H. LEE[5], P. J. DREW[2], S.-R. KOTHAPALLI[4];
[1]Pennsylvania State Univ., University Park, PA; [2]Pennsylvania State Univ., State College, PA; [3]Penn Staet, University Park, PA; [4]Penn State, University Park, PA; [5]Penn State, State College, PA; [6]The Pennsylvania State Univ., State College, PA

Abstract: Ultrasound neuromodulation is an emerging non-invasive approach for modulating neural activity and cerebral hemodynamics in vivo. However, the cellular mechanisms by which various ultrasound parameters affect specific neuronal and non-neuronal populations remain poorly understood. A key challenge is that conventional ultrasound transducers are opaque, bulky, and require large acoustic coupling media, making it difficult to integrate them with optical imaging techniques—essential tools for resolving cellular activity and neurovascular dynamics at high spatiotemporal resolution. To address this limitation, we propose an integrated multimodal optical neuroimaging and transparent ultrasound transducer (TUT) platform. The TUT has a compact form factor, allowing it to be substituted as a thinned skull cranial window, thereby enabling straightforward integration with multimodal neuroimaging setups. Using this multimodal platform, we simultaneously monitored neural and vascular responses to ultrasound stimulation with interleaved widefield calcium imaging and intrinsic optical signal imaging. We found that the TUT reliably elicited hemodynamic increases alongside modulation of neural activity. These findings underscore the potential of the TUT-integrated multimodal imaging platform in elucidating the mechanisms underlying ultrasound neuromodulation and aiding in the development of new therapeutic strategies for neurological disorders.

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Poster

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Location: SDCC Halls B-H

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Program #/Poster #: PSTR099.18/QQ17

Topic: J.08. Methods to Modulate Neural Activity

Title: Examination of Low-Intensity Focused Ultrasound Parameters in Human Motor and Somatosensory Cortices

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[1]Virginia Tech., Roanoke, VA; [2]Fralin Biomed. Res. Inst., Roanoke, VA

Abstract: Background: Low-intensity focused ultrasound (LIFU) is a noninvasive brain stimulation technique capable of modulating neural activity with high spatial precision. Although its potential for therapeutic neuromodulation is expanding, it remains unclear whether the same stimulation parameters (intensity, duty cycle, and pulse repetition frequency) produce consistent effects across different brain regions and outcome measures. Understanding whether a single parameter set has standardized effects across systems is critical for therapeutic applications of LIFU. Objective: This study aims to identify LIFU parameters that modulate excitability in both motor and somatosensory circuits, using motor evoked potentials (MEPs) and somatosensory evoked potentials (SEPs) as outcome measures. We hypothesize that LIFU parameters that demonstrate effects in the primary motor cortex will transfer to cortical somatosensory circuitry. Methods: In Arm 1, participants underwent LIFU targeted to the primary motor cortex. Eighteen pulsed parameter sets, defined by combinations of duty cycle (1%, 5%, 30%), pulse repetition frequency (10 Hz, 100 Hz, 1000 Hz), and intensity (6 W/cm², 24 W/cm²), were tested in addition to a sham condition. LIFU was applied for a 10-second duration before 20 TMS-induced MEPs with a 5-second inter-stimulus interval were recorded from the first dorsal interosseous muscle. Average MEP amplitude for each parameter set were normalized to baseline. In Arm 2, the three parameter sets that produced the largest changes in MEP amplitude alongside one non-responsive parameter set and an active sham, were applied to the primary somatosensory cortex (S1) and thalamus. Results: Preliminary analysis revealed that three LIFU parameter sets produced notable changes in MEP amplitude. The 1000 Hz, 5% duty cycle, 6 W/cm² condition reduced MEP amplitude by 65.8% (from 1032.4 ± 337.2 μV to 352.9 ± 430.9 μV). Similarly, a 10 Hz, 1% duty cycle, 24 W/cm² condition decreased MEPs by 51.0% (from 911.7 ± 414.3 μV to 446.6 ± 225.7 μV). In contrast, a 10 Hz, 30% duty cycle, 24 W/cm² condition increased MEPs by 109.4% (from 911.7 ± 414.3 μV to 1909.8 ± 761.4 μV). Conclusion: These findings suggest that specific LIFU parameters differentially effect motor excitability. The identified parameter sets will be tested in additional subjects and somatosensory regions to evaluate consistency of parameter effects across brain regions and outcome measures.

Disclosures: A. Kapoor: None. **G. Isaac:** None. **W. Legon:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.19/QQ18

Topic: J.08. Methods to Modulate Neural Activity

Title: Neuromodulation of the lateral geniculate nucleus with non-invasive transcranial ultrasound stimulation - investigations with SSVEPs and Psychophysics

Authors: *M. T. SCOTT[1], R. T. ASH[5], P. N. LIMON[2], M. MOHAMMADJAVADI[3], K. BUTTS PAULY[3], A. M. NORCIA[4];
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Abstract: Current tools to noninvasively manipulate neural circuits in the human brain are limited in spatial specificity and depth. Transcranial ultrasound stimulation (TUS) may achieve deep focal noninvasive neuromodulation. However, the efficacy of TUS, and how this relates to the TUS parameters used, is unclear. In lieu of a biophysical model, there is a need for empirical investigations of how different TUS parameters map onto neuromodulatory effects. Here, in the visual system, we present electrophysiological and psychophysical experiments designed to examine the efficacy and parameter specificity of TUS modulation. In all experiments, TUS was targeted to the left Lateral Geniculate Nucleus (LGN) during the presentation of visual stimuli. In our first experiment (N =19), we recorded steady-state visually evoked potentials (SSVEPs) to flickering stimuli. The left and right visual fields were frequency tagged, allowing the independent analysis of the sonicated hemifield and non-sonicated hemifield SSVEPs. To probe behavioral sensitivity during visual stimuli, we presented low contrast probes that participants tried to detect. Parameter specificity was probed by changing the pulse repetition frequency (PRF) across stimuli blocks (log steps from 0.49 to 4900 Hz). In a second experiment (n =10), we measured contrast detection thresholds in the sonicated hemifield, separately for 4.9Hz and 490Hz PRFs. In both experiments, the same measurements were also made when sonicating a non-LGN control site. For experiment 1, in the sonicated hemifield, our data suggest that 4.875Hz TUS to the left LGN suppressed SSVEPs relative to the non-sonicated hemifield ($p < .05$) whilst 487.5Hz TUS enhanced SSVEPs ($p < .05$). Analysis of concurrent behavioral data indicated that this was accompanied by a trend increase in the proportion of correctly detected contrast increments in the non-sonicated hemifield at 0.49Hz, 4.9Hz, and 490Hz. Conversely, for experiment 2, our data are suggestive of a significant decrease ($p < .01$) in sensitivity in the non-sonicated hemifield that is not dependent on the depth of TUS stimulation but is strongest at 490Hz. While our SSVEP work is consistent with a neuromodulatory effect that cannot be explained by an auditory confound, further work is required to characterize any link to behavioral visual sensitivity.

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Poster

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Program #/Poster #: PSTR099.20/QQ19

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH BRAIN Initiative R21EB028055
NIH R44MH131514

Title: Low-intensity pulsed ultrasound reduces the activation of microglia at the tissue electrode interface and increases chronic recording yield

Authors: *K. SNOOK[1], R. BAGWELL[1], T. D. KOZAI[2], J. GALLEGO[3], T. THAI[4], V. SINGH[4], M. L. MULVIHILL[1], J. GREASER[1];
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Abstract: Chronic neural implants hold great potential for uncovering features of neural function, treating neurological disorders, and enabling the next generation of neuroprosthetics. Research has shown that chronically implanted silicon shank electrodes often induce vascular hemorrhage, cell lysis, and tissue deformation at implantation sites, initiating a chronic injury response in both neurons and microglia. The resultant foreign body response (FBR) can cause glial scarring and neuronal cell loss near electrode sites, causing poor electrode longevity and variable functionality. Efforts such as limiting insertion damage, using flexible implants, and incorporating exogenous immunosuppressant chemicals have mitigated the FBR, but more needs done in order to clear the path for clinical applications. Recently, transcranial focused ultrasound (tFUS) was introduced as a non-invasive method to regionally modulate synaptic and non-synaptic mechanisms of neuron and glial cell activity. Research has demonstrated the neuroprotective and anti-inflammatory effects of tFUS in traumatic brain injuries and neurodegenerative disease models. Herein, we investigate the neuroprotective effects of tFUS on reducing microglia reactivity and preservation of electrophysiological measures in rodents following chronic silicon shank electrode implantation. In particular, the tFUS approach used the low-intensity pulsed ultrasound (LIPUS) principle to investigate the protective and healing effects that have been observed in models of cerebral disease and injury. To investigate the effects of tFUS on microglia activation following electrode implantation, we developed a reusable head-mounted LIPUS delivery system. Active and inactive microelectrodes were placed in the cortex of rodent models and treated with periodic LIPUS (15 min/day) or sham. Microglia reactivity and neural activity were assayed with two-photon imaging and electrophysiology recordings, respectively, over 4-7 weeks. Our results demonstrated that periodic treatment with high frequency (~1MHz) LIPUS alters microglia migration velocity and timing compared to untreated controls and that cellular encapsulation of electrodes is reduced in subjects receiving LIPUS treatment. Single unit recordings of neural activity in LIPUS treated subjects demonstrated an increase in electrode impedance, greater signal-to-noise ratios, and more identifiable single units across a greater number of electrode channels compared to controls. These results demonstrate the validity of tFUS for modulation of microglia and suppression of the FBR and provide a device for simplified non-invasive modulation.

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Poster

PSTR099: Transcranial Ultrasound Stimulation

Location: SDCC Halls B-H

Time: Sunday, November 16, 2025, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR099.21/QQ20

Topic: J.08. Methods to Modulate Neural Activity

Support: NIH; Award Number: 017237-00001

Title: Towards validation of transcranial acoustoelectric brain imaging of neuronal currents in advanced epilepsy patients.

Authors: ***P. J. C. HARRIS**[1], N. ABU FARHA[1], K. M. GOTHARD[2], R. S. WITTE[1]; [1]Med. Imaging, Univ. of Arizona, Tucson, AZ; [2]Physiol., Univ. Arizona, Col. Med., Tucson, AZ

Abstract: Scalp EEG and other noninvasive techniques designed to probe brain function are unable to sufficiently localize neuronal activity on fine spatial and temporal scales for a variety of clinical applications, such as pinpointing seizure foci and functional brain areas in refractory epilepsy patients. To address this unmet need, we are developing 4D transcranial acoustoelectric brain imaging (tABI), which depends on focused ultrasound (US) pulses to modulate tissue resistivity and noninvasively map local currents at high spatial resolution.The goals of this study are to 1) develop tABI instrumentation for conducting feasibility studies in human subjects and 2) establish a behavioral paradigm for validating tABI in advanced epilepsy patients. First, we implemented a human head model for ex vivo validation and optimization of tABI using controlled time-varying currents generated by an electrode array embedded in a conductive agarose gel inside a skull. A neuronavigation system helped guide placement of a 1.5D 0.6 MHz US array on the skull for mapping deep current sources. To perform tABI scans, an US beam was rapidly steered and focused through the skull as electrodes on the skull recorded both standard surface potentials (<10 kHz) and high frequency (MHz) interaction signals. Second, we designed a behavioral task to activate the primary motor cortex in retractable epilepsy patients implanted with subdural strip electrodes for extraoperative monitoring. Three subjects were asked to pump a ball (30 sec pumping or resting X 4 on each hand) while recording invasive EEG. One subject performed the task inside a 3 T Skyra MRI scanner to generate fMRI maps of the hand area. We successfully performed 4D tABI scans of deep currents (0.2 - 10 mA) in the

ex vivo head model with a spatial resolution <5 mm and temporal sampling of 0.25ms while also collecting physiologic signals and surface potentials. In epilepsy patients with invasive EEG, we observed neural activity (1.17 ± 0.19 Hz) highly coherent with the frequency of the hand pumping task (1.24 ± 0.11 Hz). While the strip electrodes over primary motor cortex (lateral frontal and orbital frontal) elicited the strongest response, we noted stark differences in overall brain responses (higher amplitude, more contacts in coherence) between left- and right-hand pumping. There was also a smaller representation of right-hand pumping compared to the left on fMRI, corresponding to left temporal lobe epilepsy in this patient. These results support further translation of tABI technology as a new modality for high resolution imaging of neuronal currents with initial validation in epilepsy patients and ultimately healthy volunteers.

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Poster

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Topic: J.04. Physiological Methods

Support: NIH U01 EB028662
Flinn Foundation, 23-06543

Title: Transcranial Acoustoelectric Imaging of Neuronal Currents: Progress and Challenges

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Abstract: A broad spectrum of brain disorders—from epilepsy to depression—are characterized by abnormal electrical conduction pathways. Although scalp EEG is the gold standard for electrical brain mapping, it suffers from poor spatial resolution and often inadequate for a variety of applications that depend on accurate mapping of neuronal currents. To address this unmet clinical and research need, we are developing 4D transcranial acoustoelectric brain imaging (tABI), a disruptive technology in which an ultrasound beam is rapidly steered and focused in the brain while recording weak AE interaction signals (or “sparks”) to noninvasively map local currents on the scale of millimeters and milliseconds. This presentation will describe recent progress and challenges developing tABI in both *ex vivo* and *in vivo* preparations as we transition towards feasibility studies in humans. First, we demonstrate 4D tABI in a human head model with real skull containing a conductive gel (1% Agarose, 0.9% NaCl) and an embedded depth electrode array for generating controlled, arbitrary sources and sinks. The prototype system

employs a 2D 0.6 MHz US array for focusing and steering a beam through thick skull, surface electrodes for recording both EEG (<10 kHz) and AE (>200 kHz) signals, and a neuronavigation system with imported MRIs for guiding placement of the US probe. Second, we have developed an acute swine model for evoking neuronal activity in the somatosensory cortex or hippocampus, and performing 4D tABI through the thinned pig skull. These preclinical models demonstrate that tABI is capable of volumetric mapping of fast, time-varying currents less than 500 μA through bone highly correlated with the directly measured currents (R>0.95) with millimeter accuracy and resolution. Ongoing efforts are focused on several strategies to dramatically improve the detection limit towards mapping weak neuronal currents <<100 μA as we progress towards feasibility studies in refractory epilepsy patients and healthy volunteers.

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Poster

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Topic: J.08. Methods to Modulate Neural Activity

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Title: Ultrasound-guided bioluminescent optogenetics enables spatially targeted neuromodulation of distributed brain circuits noninvasively

Authors: *E. MURPHY[1], M. DOYLEY[1], J. F. MITCHELL[2], K. H. WANG[3], M. GOMEZ-RAMIREZ[4];
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Abstract: Chemogenetic tools such as DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) have revolutionized systems neuroscience by enabling targeted, cell-type-specific modulation of neural circuits using a noninvasive approach. However, most chemogenetics methods (e.g., DREADDs) are limited by several key factors such as slow onset and offset kinetics, poor temporal precision, and concerns over off-target effects due to ligand metabolism in the case of DREADDs (e.g., CNO to clozapine). BioLuminescent-OptoGenetics (BL-OG) is a next-generation hybrid chemo- and opto-genetic method that overcomes many of these limitations by using luciferase-driven bioluminescence to activate tethered opsins, offering faster temporal control, no known off-target effects, and an intrinsic optical readout of

neuromodulation strength. However, a current major limitation of chemogenetic methods, including the current approach for BL-OG, is their lack of spatial precision. A single systemic injection of the substrate modulates all brain regions expressing the activating molecule resulting in all-or-none activation. This global activation makes it difficult to dissect the function of individual nodes within distributed networks, a significant challenge when studying complex behaviors that rely on coordinated activity across widespread but functionally distinct brain areas. To address this issue, we combine BL-OG with focused ultrasound (FUS) to transiently open the blood-brain barrier (BBB), enabling localized delivery of diluted coelenterazine (CTZ). Specifically, we applied FUS to the primary somatosensory (SI) cortex of mice to open the BBB in that region and systemically injected a diluted amount of h-CTZ, thereby restricting its bioavailability to the SI cortex. Using bioluminescence and 2-photon calcium imaging, we showed increased BL-OG effects in brain areas targeted with FUS. These data demonstrate that our combined FUS and BL-OG method can generate site-specific neuromodulation within large-scale brain networks in a noninvasive manner.

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Poster

PSTR099: Transcranial Ultrasound Stimulation

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Program #/Poster #: PSTR099.24/RR3

Topic: J.08. Methods to Modulate Neural Activity

Title: Optimized focused ultrasound neuromodulation of brain reward center activity and behavior

Authors: *C. E. LUFF[1,2], K. MURPHY[1], O. C. GONZALEZ[1], J. MORGA-RODRIGUEZ[1], J. A. KAUER[1], L. DE LECEA[1];
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Abstract: Substance Use Disorder (SUD) is a chronic brain disease. Most drugs of abuse exert their initial reinforcing effects by activating meso-cortico-limbic dopaminergic (DA) reward circuits in the brain, including the ventral tegmental area (VTA)- a key source of DA projections to reward-related regions such as the nucleus accumbens (NAc). At the circuit level, VTA DA activity is essential for reinforcing reward, attributing motivational salience to stimuli, and computing reward prediction error. Thus, modifying the output of these circuits by selectively stimulating or inhibiting different cell-types in the context of addictive substances, offers the opportunity for dissociating these cues from their rewarding component. Neuromodulation techniques that can target these circuits with high specificity offer therapeutic potential for SUD. Transcranial focused ultrasound (fUS) is an emerging, non-invasive technology capable of stimulating deep brain regions with millimeter precision, and holds promise for treating SUD. There is, however, no consensus on the ideal fUS parameters for either stimulating or inhibiting

neuronal activity, largely due to the large fUS parameter space and brain heterogeneity. Accordingly, the optimal parameters for fUS-induced modulation of DA activity in the VTA are unknown. To address these questions, we developed PhoCUS (Photometry-Coupled Ultrasound System), which enables simultaneous fUS, and fiber photometry readout of neuronal calcium activity of genetically defined neuronal subtypes, in freely behaving mice. In this study we aimed to answer the following questions: What parameters most effectively inhibit and activate DA neuronal activity in the VTA, either directly, or via activation or inhibition of local inhibitory GABAergic neurons? Does optimized fUS neuromodulation of this region lead to modulation of reward-related behavior? Using the PhoCUS system, we identified optimized fUS stimulation parameters that reliably elicit bidirectional (excitatory and inhibitory) modulation of VTA DA activity across temporal scales. Additionally, these parameters were unique to the VTA, suggesting a cell-type or network specific effect at play. Optimized parameters for DA inhibition were perceived as aversive, as demonstrated by real time place preference (RTPP) experiments, suggesting that optimized fUS can functionally alter reward circuit activity. Together, this work provides a foundation for dissecting network-level mechanisms of ultrasound neuromodulation, and guiding rational development of fUS-based interventions for neuropsychiatric and neurological disorders, including SUD.

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Poster

PSTR099: Transcranial Ultrasound Stimulation

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Program #/Poster #: PSTR099.25/RR4

Topic: J.08. Methods to Modulate Neural Activity

Title: Supra- vs. Subharmonic Cavitation Signals as Indicators for Feedback-Controlled FUS BBB Modulation in Non-Human Primates

Authors: *S. HAN, W. CHANG;
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Abstract: Efficient, controlled focused ultrasound (FUS)-induced blood-brain barrier opening (BBBO) is vital for central nervous system (CNS) therapeutic delivery. This study tested if closed-loop FUS, with real-time passive cavitation detection (PCD) signal interpretation controlling emissions to the inertial cavitation (IC) threshold, yields more efficient and safe BBBO versus stable cavitation-only methods. An integrated FUS system enabling this cavitation-threshold feedback was used in an NHP model. After IV microbubble administration, multiple intracranial targets in one cynomolgus monkey were sonicated. FUS power was precisely feedback-controlled via an Acoustic Cavitation Indicator (ACI) using real-time PCD signal interpretation to identify cavitation near IC threshold, aiming for enhanced efficacy. Post-

sonication MRI confirmed successful BBBO at all targeted locations. The ACI effectively tracked cavitation states and correlated strongly with both BBBO volume and MR contrast enhancement. This strategy yielded substantial BBBO without significant adverse effects (MRI/behavioral assessment), suggesting controlled operation near IC threshold safely improves BBBO efficiency. This validates PCD-guided, IC-threshold feedback as a more efficient, safe FUS-BBBO method in large animals, pivotal for clinical translation. These achievements were attained with a single-element transducer FUS system. This configuration, offering precise focal targeting and a simplified acoustic field/PCD signal interpretation environment, was instrumental for rigorously validating the proposed advanced cavitation control algorithm—controlling acoustic energy to the IC threshold via precise PCD signal interpretation. The core significance of this study lies in demonstrating that such precise control with a single-element system potentially enables more efficient, safer BBBO than targeting SC alone, and in presenting its specific cavitation control methodology. Thus, this study contributes to FUS-BBBO technology advancement in applications requiring precise focal control, providing foundational data and effective control strategies for understanding and managing cavitation phenomena in various future FUS systems.

Disclosures: S. Han: None. **W. Chang:** None.

Poster

PSTR099: Transcranial Ultrasound Stimulation

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Topic: J.08. Methods to Modulate Neural Activity

Support: KAKENHI JP23K14312

Title: Trpc6 ion channels mediate neuronal activation induced by ultrasonic acoustic radiation force

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Abstract: Ultrasound is widely employed in clinical applications, including diagnostic imaging and the treatment of fractures and chronic pain, owing to its non-invasiveness, deep tissue penetration, and high spatiotemporal resolution. Although ultrasound has been shown to modulate neuronal activity in both the central and peripheral nervous systems, the specific bioeffects responsible for this activation remain poorly understood. To address this fundamental question, we established an in vitro experimental system that enables real-time Ca^{2+} imaging to visualize neuronal activity during ultrasound irradiation. Using this system, we confirmed that ultrasound induces neuronal activation and investigated which specific bioeffects are critical for this activation. In our experimental setup, cultured mouse cortical neurons were activated by ultrasound at a frequency of 1 MHz, a pulse repetition frequency of 100 Hz, and a 50% duty

cycle. A circular pressure distribution with peak pressure at the center was observed when ultrasound was measured in free water using a needle-type hydrophone. Ultrasound exerts several biophysical effects, including thermal effects, cavitation, and acoustic radiation force. Under our stimulation parameters, no significant temperature rise or evidence of cavitation was observed, indicating that these effects are unlikely to account for the neuronal activation. Instead, our findings suggest that acoustic radiation force plays a key role in mediating Ca^{2+} transients in neurons. Importantly, pharmacological and genetic experiments revealed that ultrasound-induced neuronal activation depends on extracellular Ca^{2+} influx, the generation of action potentials, and functional network activity. Furthermore, we found that TRPC6 ion channels play an essential role in mediating ultrasound-induced neuronal activation both in cultured neurons and in the living mouse brain. Taken together, our findings identify TRPC6 as a critical component of ultrasound-induced neuronal responses in the mammalian brain, shedding light on the molecular and cellular mechanisms of ultrasound neuromodulation and providing its future development for clinical applications.

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